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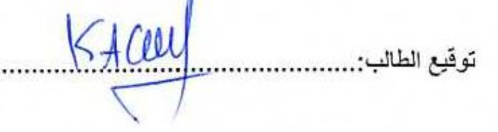
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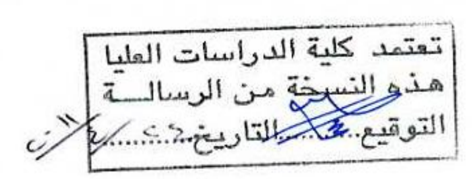
نموذج رقم (١٨)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية
وقوانين الجامعة الأردنية وأنظمتها وتعليماتها
لطلبة الماجستير

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تخصص: الصيدلة السريرية الكليـــــة: الصيدلة

عنوان الرسالة: Evaluation of Prolonged Intermittent Fasting Effect on Immune System and Some Blood Inflammatory cytokines in Healthy Volunteers

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير عندما قمت شخصيا" باعداد رسالتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الرسائل العلمية. كما أنني أعلن بأن رسالتي هذه غير منقولة أو مستلة من رسائل أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

توقيع الطالب:  التاريخ: 24 / 04 / 2011


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EVALUATION OF PROLONGED INTERMITTENT FASTING EFFECT ON IMMUNE SYSTEM AND SOME BLOOD INFLAMMATORY CYTOKINES IN HEALTHY VOLUNTEERS

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This Dissertation was Submitted in Partial Fulfillment of the Requirements for the Master's Degree in Clinical Pharmacy

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April, 2011

COMMITTEE DECISION

This Thesis/Dissertation (Evaluation of Prolonged Intermittent Fasting Effect on Immune System and Some Blood Inflammatory Cytokines in Healthy Volunteers) was Successfully Defended and Approved on 18/04/2011.

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DEDICATION

With Love and Faithfulness, I Dedicate this Work to
My parents, sisters and Brothers
To
My husband Dr. Mahfoud Ikhenache
To my supervisors Dr Mohammad and Dr. Yasser
To my beloved sister Amel who always stood beside me
And to all of my teachers and friends

For giving me their support and the power to proceed.

Acknowledgments

It is my pleasure to express my deepest gratitude and sincere thanks to my supervisors, Dr. Mohammad Khalil Mohammad and Dr. Yasser Khalil Bustanji for their continuous guidance throughout my work. Special thanks are also given to Dr. Moez Al-Islam for his help.

My deepest and sincere thanks are presented to all members of the examination committee.

I also would like to thank my husband Dr. Mahfoud Ikhanache for his support and patience.

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List of Abbreviations

ADF	Alternate Day Fasting
al	*Ad Libitum*
Apo	Apolipoprotein
APR	Acute-Phase Response
BDNF	Brain-Derived Neutrophic Factor
BG	Blood Glucose
BMI	Body Mass Index
CBC	Complete Blood Count
CD	Cluster of Differentiation
CHD	Coronary Heart Diseases
CR	Caloric Restriction
CRP	C-Reactive Protein
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
F	Female
FBG	Fasting Blood Glucose
GP	Glycoprotein
Hp	Haptoglobin
HbA1C	Glycosylated Hemoglobin

HC	Hip Circumference
Hc	Homocysteine
HDL	High Density Lipoprotein
IF	Intermittent Fasting
IGF-1	Insulin Like Growth Factor-1
IL	Interleukin
IL-1 RI	Type 1 IL-1 Receptor
IL-1 RII	Type 2 IL-1 Receptor
IL-1ra	Interleukin-1 receptor antagonist
kDa	Kilo Dalton
LDL	Low Density Lipoprotein
Lep.	Leptin
LPL	Lipoprotein Lipase
M	Male
MDA	Malondialdehyde
mRNA	Messenger Ribonucleic Acid
NF-$_{K}$B	Nuclear Factor –Kappa B
NIDDM	Noninsulin-Dependent Diabetes Mellitus
NK	Natural Killer
O.D	Optical Density
PBS	Phosphate-Buffered Saline
RIF	Ramadan Intermittent Fasting
ROS	Reactive Oxygen Species
rpm	Round Per Minute

SAA	Serum Amyloid A
SBP	Systolic Blood Pressure
SD	Standard Deviation
SPSS	Statistical Package For Social Sciences
SQ	Subcutaneous
sTNF-α	Soluble Tumor Necrosis Factor-α
TACE	TNF-α Converting Enzyme
TC	Total Cholesterol
TG	Triglyceride
TNF	Tumor Necrosis Factor
VLDL	Very Low Density Lipoprotein
WBC	White Blood Cells
WC	Waist Circumference

EVALUATION OF PROLONGED INTERMITTENT FASTING EFFECT ON IMMUNE SYSTEM AND SOME BLOOD INFLAMMATORY CYTOKINES IN HEALTHY VOLUNTEERS

By
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ABTRACT

Long-lasting modification in meal frequency during intermittent fasting may have beneficial effects on inflammatory cytokines, as well as on immune cells and anthropometric parameters. We aimed to investigate the effects of prolonged intermittent fasting practiced during Ramadan on circulating immune cells and inflammatory cytokines. This study was carried out during Ramadan 2009. Fifty healthy volunteers (21 males aged between 18 and 49 years, and 29 females aged between 18 and 51 years) from Jordanian population participated in this study. Blood sampling was drawn one week before Ramadan, on the third week of Ramadan, and one month after the end of Ramadan. Total white blood cells (WBC) count, differential WBC count, serum interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α were evaluated for each study time-point. Height, weight, body mass index (BMI), body fat percentage, waist and hip circumferences, and systolic and diastolic blood pressures were also measured during each of the three visits. Significant weight reduction, significant decrease in BMI, and significant decrease in both systolic and diastolic blood pressures were observed during Ramadan, compared to before Ramadan values. Circulating IL-1β, IL-6, and TNF-α levels were significantly reduced during Ramadan. Total WBC, lymphocytes, granulocytes, and monocytes counts, being in the reference ranges, were also significantly decreased during Ramadan. Our results clearly indicate that prolonged intermittent fasting practiced during Ramadan has some positive effects on the inflammatory status of the body.

1. INTRODUCTION

The development of an effective immune response involves the intervention of lymphoid cells, inflammatory cells, and hematopoietic cells. The complex interactions among these cells are mediated by a group of proteins collectively designed as cytokines (Goldsby, et al., 2002). Immune reactions are complex, changes in one component could affect several others; this is illustrated in the cytokine network theory, where alteration of the concentration of one cytokine will lead to a cascade of effects on others (Parkin and Cohen, 2001).

Notably, a major concern is that excessive caloric intake and subsequent obesity are characterized by a chronic state of low-grade inflammation, including high circulating proinflammatory cytokine levels (Fontana, et al., 2007; You, et al., 2005). In addition, there is an accumulating evidence that chronic inflammation plays a key role in the initiation and progression of atherosclerosis (Haddy, et al., 2003; Lind, 2003), insulin resistance (Spranger, et al., 2003; Muller, et al., 2002), cardiovascular diseases (CVD) (Sciarretta, et al., 2007), and tissue damage associated with many types of cancer (Schafer and Brugge, 2007; Federico, et al., 2010). This state of low-grade chronic systemic inflammation is detected by elevations of inflammatory biomarkers ranging from leukocytes to acute phase reactants such as C-reactive protein (CRP) (Schmidt, et al., 2005).

Over the past few years, an increasing number of physiological effects of caloric restriction (CR) and intermittent fasting (IF) have been documented in studies on rodents, monkeys and humans. Prominent among these are increased lifespan (Varady and Hellerstein, 2007), decreased mortality from cancers and CVD (Ahmet, et al., 2005; Longo and Fontana, 2009; Wan, et al., 2010), improved insulin sensitivity (Argentino, et al.,

2005), and reduced oxidative stress and inflammation (Castello, et al., 2010). Interestingly, in several epidemiologic and experimental studies, the inflammatory biomarkers such as interleukin-6 (IL-6) and CRP were found to be significantly depressed by CR and IF (Brannon, et al., 2009; Aksungar, et al., 2007).

One of the most important rules of Islam is that any healthy adult Muslim must fast from down to sunset during the holy month of Ramadan. Over the past forty years, emerging evidence from epidemiologic studies supports the health-related benefits of Ramadan intermittent fasting (RIF), including improved insulin sensitivity (Shariatpanahi, et al., 2008), decreased atherogenic risk (Aksungar, et al., 2005), decreased oxidative stress (Ibrahim, et al., 2008), and decreased inflammation (Aksungar, et al., 2007; Unalacak, et al., 2011).

Although there is an evidence linking IF with decreased inflammatory biomarkers (Brannon, et al., 2009; Ugochukwu and Figgers, 2006), few reports have examined the effect of long lasting modification of food intake during RIF on circulating inflammatory cytokines (Aksungar, et al., 2007; Unalacak, et al., 2011). Therefore, our study aimed to evaluate the anti-inflammatory effect of prolonged intermittent fasting practiced during the holy month of Ramadan, in healthy Jordanian volunteers. Based on the previously mentioned reduced inflammation, cancer, and CVD risk in those who practiced CR and IF, we hypothesized that healthy fasting individuals practicing RIF would have decreased circulating proinflammatory cytokine levels during RIF than during non fasting periods. Accordingly, we have examined the effect of RIF on serum levels of proinflammatory cytokines, including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. The effects of RIF on leukocytes differential count, anthropometric characteristics, and blood pressure, were also determined.

2. LITERATURE REVIEW

2. 1. Assessment of the Immune System

The immune system is a complex and highly regulated network of innate (natural) immunity and adaptive (acquired) immunity (Chaplin, 2003). The innate (non-specific) immunity more usually encompasses the elements of the immune system (neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins) which provide immediate host defense. Whereas, adaptive immunity consists of antigen-specific reactions through T lymphocytes and B lymphocytes (Parkin and Cohen, 2001). The recruitment of these immune cells to the site of inflammation is achieved by the interaction of cellular receptors (adhesion molecules) and external factors (cytokines) (Chaplin, 2003).

Clinical assessment of immunity requires the investigation of the four major components of the immune system that participate in host defense and in the pathogenesis of autoimmune diseases: (1) humoral immunity (B cells); (2) cell-mediated immunity (T cells); (3) phagocytic cells of the reticuloendothelial system (macrophages), as well as polymorphonuclear leukocytes (granulocytes); and (4) complement (Haynes, et al., 2010). The quantification of lymphocytes, monocytes, and granulocytes are usually assessed in clinical practice by means of leukocytes differential count.

2. 2. Inflammatory Response

Elimination of foreign antigen by cellular or humoral processes of innate immune system is integrally linked to the inflammatory response (Nathan, 2002). This latter induces a sequence of reactions initially involving cytokines, neutrophils, adhesion molecules, complement, and immunoglobulins. Later, monocytes and lymphocytes are involved (Barret, et al., 2010). Acute inflammatory response is characterized by the rapid induction

of an oxidative state as a means of non specifically combating foreign antigens. This response is called acute-phase response (APR) (Elgert, 1996). During the APR, some proteins are increased (positive acute-phase reactants) such as CRP and some high density lipoprotein (HDL)-associated proteins like serum amyloid A (SAA), whereas others are decreased (negative acute-phase reactants) such as apolipoprotein A-I (Apo A-I) (Navab, et al., 2005). Alternatively, uncontrolled lymphocyte activation and unregulated antibody production can lead to persistent or chronic inflammation responsible for tissue damage and organ dysfunction (Nathan, 2002).

Chronic low-grade systemic inflammation has been introduced as a term for conditions in which a typically two to threefold increase in the systemic concentrations of TNF-α, IL-1, IL-6, and CRP is reflected (Petersen and Pedersen, 2005). This low-grade inflammation can begin very early in life, and is present in an important fraction of children, especially those who are obese (Schmidt and Duncan, 2003). Recent evidence has associated visceral obesity with chronic low-grade inflammation, as resulting from chronic activation of the innate immune system (Bastard, et al., 2006). It is expected that even this low-level chronic inflammation would have pleiotropic effects on the function of many immune cells and on tissues, and that it should be possible to develop strategies to dampen this chronic inflammation (Swain and Nikolich-Zugich, 2009).

Evidence is accumulating that a transcription factor, nuclear factor-$_{K}$B (NF-κB), plays a key role in the inflammatory response (Christman, et al., 2000). This factor normally exists in the cytoplasm of cells bound to its inhibitory protein $I_{K-}B_{\alpha}$, which renders it inactive (Roman-Blas and Jimenez, 2006). The activation of NF-$_{k}$B involves the intervention of several stimuli separating NF-$_{K}$B from $I_{K-}B_{\alpha}$, which is then degraded. Examples of such stimuli are reactive oxygen species (ROS) and oxidants (Portugal, et al.,

2007). Following its activation, NF-$_{k}$B translocates into the nucleus, where it activates its target genes such as proinflammatory cytokine genes (Portugal, et al., 2007).

2. 3. Inflammatory Biomarkers

2. 3. 1. C-reactive protein

C-reactive protein (CRP) is a pentameric protein synthesized in the liver. It is a marker of the acute phase response which is part of the innate immune system. Its main action is to activate complement and to counteract infections. C-reactive protein may mediate endothelial dysfunction and the promotion of thrombosis via activation and co-localization of complement and up-regulation of monocytes to produce progoagulant tissue factor (Lagrand, et al., 1997). In addition, CRP directly inhibits the production of nitric oxide which could impair the response to ischemia (Hamdy et al., 2008). The main stimuli for secretion of CRP are IL-1 and IL-6, and indirectly also TNF-α (Hamdy, et al., 2008). Circulating levels of CRP have been found to be related to a number of well known CVD risk factors, such as obesity, smoking, serum fibrinogen, HDL cholesterol, heart rate, blood pressure, serum triglycerides (TG), fasting blood glucose (FBG), and apolipoprotein B (Apo B) (Cook, et al., 2000; Mendall, et al., 1996).

2. 3. 2. Fibrinogen

This large protein is created by the liver. It affects homeostasis, increases blood viscosity and leukocytes adhesion (Magliano, et al., 2003). Its secretion is up-regulated by a number of various cytokines, and is related to number of cardiovascular risk factors such as smoking (Lind, 2003). Fibrinogen levels increase with age, total cholesterol (TC), low density lipoprotein (LDL)-cholesterol, and TG, and are also higher among the obese, smokers and people with hypertension and diabetes mellitus (DM) (Genest and Cohn, 1995).

2. 3. 3. Serum amyloid A

Serum amyloid A (SAA), a major acute phase reactant, is a liver-derived HDL-associated apolipoprotein (Urieli-Shoval, et al., 2000). In inflammatory conditions, the production of SAA from the liver increases dramatically under the influence of IL-6, IL-1, and TNF-α (Uhlar and Whitehead, 1999). Serum amyloid A is a multifunctional protein involved in cholesterol transport and metabolism, and in modulating the inflammatory response via anti- and proinflammatory activities. Indeed, SAA was proposed as a signal for redirecting HDL to sites of tissue destruction and cholesterol accumulation (Urieli-Shoval, et al., 2000).

2. 3. 4. Leukocyte count

Leukocyte count refers to the total number of white blood cells (WBC) in peripheral blood. Leukocytes have a number of effects, such as antibody and cytokine-synthesis and direct effects on microorganisms (Goldsby, et al., 2002). Various cytokines affect the number of leukocytes in peripheral blood. Also the leukocyte count has been related to different CVD risk factors, such as smoking, obesity and blood pressure (Lind, 2003).

The leukocytes differential count has many components, and for assessing the immune system, it is important to know that the different immune cells are present. These include lymphocytes, monocytes, and granulocytes (Elgert, 1996). They represent 25-45%, less than 10%, and 45-75% of the total white blood cells, respectively (Goldsby, et al., 2002).

Lymphocytes mediate the defining immunologic attributes of specificity, diversity, memory, and self/non self recognition (Goldsby, et al., 2002). Lymphocytes are subdivided into two major populations; B and T cells. After activation, B cells differentiate into plasma cells producing specific immunoglobulins. T cells, in turn, are subdivided into helper and cytotoxic cells (Goldsby, et al., 2002).

Monocytes circulate in the blood or reside in a spleen reservoir before entering tissue and giving rise to macrophages or dendritic cells. They mediate essential functions of innate immunity, including phagocytosis and cytokine production (Robbins and Swirski, 2010).

Granulocytes are classified as neutrophils, eosinophils, or basophils (Goldsby, et al., 2002). Neutrophils (45-70%), much more numerous than eosinophils (1-3%) or basophils (<1%), are active phagocytic cells which increase dramatically in response to infections (Goldsby, et al., 2002).

2. 3. 5. Cytokines

Cytokine is a term used to describe a broad range of structurally diverse molecular families and individual proteins best known for their many roles in cell-mediated immunity and inflammatory reactions (Goldsby, et al., 2002). Their functions are mediated by binding specific receptors and their activities include regulating cell activation, hematopoiesis, apoptosis, and cell proliferation (Johnston and Webster, 2009). Leukocytes are the primary source of cytokines, although they may be produced by many other cell types as well (Parkin and Cohen, 2001).

Cytokines act at picolmolar to nanomolar concentrations on cytokine receptors expressed by target cells (Elgert, 1996). High concentrations of cytokines will commonly cause shedding of the receptors for the cytokines from cell surfaces, thus reducing further responses (Parkin and Cohen, 2001). The release of surface receptors may down regulate the receptors, thus reducing cell responsiveness, with circulating receptors acting as a buffer for the free cytokine in circulation (Van Zee, et al., 1992).

2. 3. 5. 1. Proinflammatory cytokines

According to their role in infection and/or inflammation, cytokines have been divided into two main groups (Dinarello, 2000). Some cytokines clearly promote inflammation and are called proinflammatory cytokines (e.g. IL-1β, IL-8, IL-6, and TNF-α), whereas other cytokines suppress the activity of proinflammatory cytokines and are called anti-inflammatory cytokines (e.g. IL-1 receptor antagonist and IL-10) (Dinarello, 2000). The proinflammatory cytokines form part of a complex defense network that protects the host against inflammatory agents and injury. However, overproduction of these cytokines may harm the host by inducing tissue injury or alteration of the immune system. Indeed, abnormal expression of proinflammatory cytokines may contribute to generalized auto-immune diseases such as rheumatoid arthritis (Ugochukwu and Figgers, 2006). Here we will discuss the three proinflammatory cytokines investigated in our study (IL-1β, IL-6, and TNF-α).

2. 3. 5. 1. 1. Interleukin (IL)-1β

Interleukin-1 is a name that designates two proteins, IL-1α and IL-1β, that are the products of distinct genes, but recognize the same cell surface receptors (Johnston and Webster, 2009). Most of IL-1 activity in circulation comes from IL-1β (Elgert, 1996). Interleukin-1 has also been known by a number of alternative names, including lymphocyte activating factor, endogenous pyrogen, catabolin, hemopoietin-1, melanoma growth inhibition factor, and osteoclast activating factor (Elgert, 1996).

Two distinct IL-1 receptor binding proteins have been identified. These are termed type 1 IL-1 receptor (IL-1 RI) and type 2 IL-1 receptor (IL-1 RII) (Svenson, et al., 1993).

The effects of IL-1 are not limited to inflammation, as IL-1 has also been associated with bone formation and remodeling (Kusano, et al., 1998), insulin secretion (Mandrup-

Poulsen, 1994), appetite regulation (Plata-Salaman, 1996), and sleep regulation (Krueger, et al., 2001).

Interleukin-1 was found to be released by a wide variety of cells including astrocytes, adrenal cortical cells, macrophages, monocytes, endothelial cells, keratinocytes, platelets, neurons, neutrophils, osteoblasts, fibroblasts, and Langerhans cells (Elgert, 1996).

At low locally produced levels, IL-1 enhances CD4+T cell proliferation, promotes B cell growth and differentiation, autocrinelly stimulates IL-1 production, induces IL-6 synthesis, and enhances leukocyte/endothelial cell adhesion. At high levels, IL-1 enters the circulation and causes fever, stimulates the release of acute phase proteins from cells and induces cachexia (Elgert, 1996). Interleukin-1β and TNF-α are the earliest mediators of the acute phase proteins. Both cytokines induce a second wave of cytokines, including IL-6 and chemokines (Bruunsgaard, et al., 2000). Although IL-1 enhances immune defense functions, it also triggers inflammation and tissue damage. To counter IL-1 activity, monocytes and macrophages produce IL-1 receptor antagonist (IL-1ra) that blocks IL-1 activity by binding to IL-1 receptors, thus functioning as a competitive inhibitor of IL-1 (Elgert, 1996).

2. 3. 5. 1. 2. Tumor Necrosis Factor (TNF)-α

Tumor necrosis factor-α is a pleiotropic cytokine, in that it mediates a wide variety of biologic activities. Some activities of TNF-α are common to a variety of diseases, such as those that modulate cell recruitment, cell proliferation, cell death and immune regulation. Other biologic activities of TNF-α may be restricted to certain diseases, such as matrix degradation and osteoclastogenesis in rheumatoid arthritis or granuloma formation in Crohn's disease (Tracey, et al., 2008).

TNF-α has not only anti-tumoral activities, but also, and mainly, immunomodulatory, proinflammatory, and metabolic actions (Malik and Balkwill, 1988). Depending on its level and site of secretion, TNF-α can either play an advantageous or a damaging role. At low concentrations in tissues, TNF-α is thought to have beneficial effects, such as the augmentation of host defense mechanisms against infections. At high concentrations, TNF-α can lead to excess inflammation and organ injury (Tracey, et al., 2008).

A schematic view of the network of ligands, receptors and signaling pathways that encompass TNF-α biology is shown in Figure 2.1 which illustrates several layers of complexity. First, TNF-α is released from cells as a soluble cytokine (sTNF-α) after being enzymatically cleaved from its cell surface–bound precursor (tmTNF-α) by TNF-α-converting enzyme (TACE). Both sTNF-α and tmTNF-α are biologically active, and the relative amounts of each are collectively determined by the inducing stimuli, the cell types involved, the activation status of the cells, the amounts of active TACE and the amounts of natural TACE inhibitors, such as tissue inhibitor of metalloproteinase-3 (Smookler, et al., 2006). Receptor-mediated effects of sTNF and tmTNF can lead alternatively to activation of NF-κB or to apoptosis, depending on the metabolic state of the cell.

Many different immune and non immune cell types can produce TNF-α, including macrophages, T cells, mast cells, granulocytes, natural killer (NK) cells, fibroblasts, neurons, keratinocytes and smooth muscle cells. The production of TNF-α in macrophages can be induced by a wide variety of stimuli, including bacteria, viruses, immune complexes, IL-1,complement factors, tumor cells, irradiation, ischemia/hypoxia and trauma (Tracey, et al., 2008).

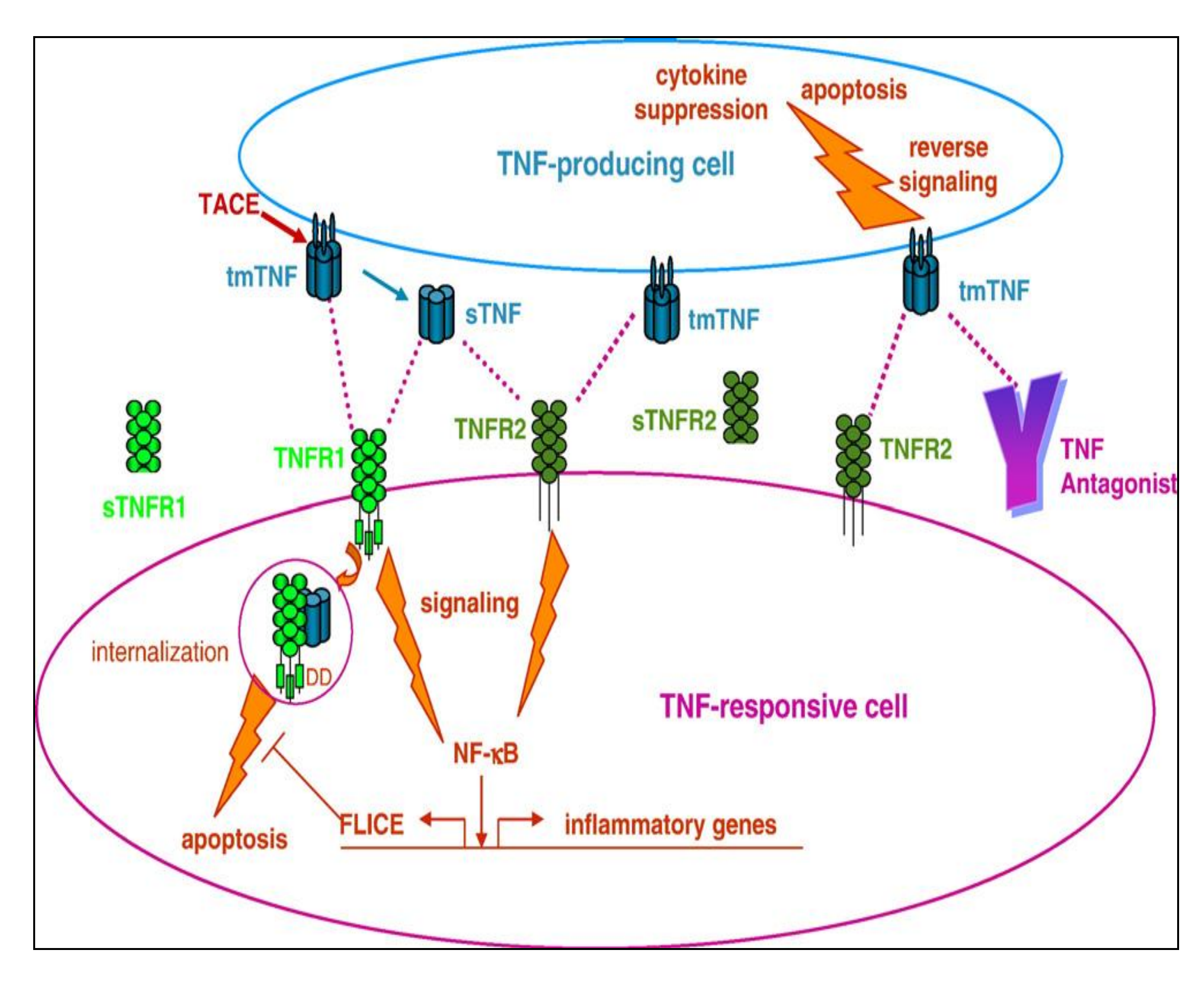


Figure 2.1. Biology of TNF-α Production, Receptor Interaction and Signaling (From : Tracey, et al., 2008).

2. 3. 5. 1. 3. Interleukin (IL)-6

Interleukin-6 was isolated under a variety of names, including interferon β2, IL-1 inducible 26 kD protein, hepatocyte stimulating factor, cytotoxic T-cell differentiation factor, and B-cell differentiation factor (Kishimoto, 1989). Interleukin-6 has been described as both pro-inflammatory and anti-inflammatory molecule, a promoter of hematopoiesis, and an inducer of plasma cell development and bone remodeling (Barton, 1997).

Interleukin-6 may be produced by many different cells in response to IL-1 and TNF-α (Johnson and Webster, 2009). In fact, cells known to express IL-6 include CD8+ T cells, fibroblasts, adipocytes, osteoclasts, megacaryocytes, endothelial cells, retinal pigment cells, mast cells, keratinocytes, astrocytes, pancreatic islet β cells, granulocytes, B cells, vascular smooth muscle cells, macrophages, neurons, and hepatocytes (Kishimoto, 1989).

Interleukin-6 exerts growth-inducing, growth inhibiting, and differentiation-inducing effects, depending on the target cells. For example, it is a T-cell activation, proliferation, and differentiation factor, as well as the main growth factor for B cells late in their differentiation. Interleukin-6 also induces APR in liver cells, growth promotion of myeloma/plasmocytoma/hybridoma cells, inhibition of certain myeloid leukemic cells, and induction of their differentiation to macrophages (Elgert, 1996).

The biological activities of IL-6 are initiated by binding to a high-affinity receptor complex, consisting of two membrane glycoproteins (GP), GP 80 and GP 130. It has been suggested that elevated levels of IL-6 are associated with increased production of IL-6 receptors. In addition, soluble form of GP 130 may have antagonistic properties (Mohamed-Ali, et al., 1998).

2. 3. 5. 2. Proinflammatory cytokine abnormalities in human diseases

2. 3. 5. 2. 1. Auto-immune diseases

The proinflammatory cytokines investigated in our study have been shown to play an important role in the pathogenesis of many immune-mediated inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, and psoriasis. In addition, many cytokine antagonists are now registered in many countries and being used in the management of the above mentioned abnormalities (Donnelly, et al., 2009). Today, there are three registered TNF-α antagonists in the United States and the European Union: Infliximab, Etanercept and Adalimumab; each is indicated for several immune-mediated inflammatory diseases. Infleximab (Remicade®) is a laboratory produced antibody that targets TNF-α. Etanarcept (Enbrel®) is a soluble receptor for TNF-α. Adalimumab (Humira®) is a TNF-α receptor bound to monoclonal antibody. All these drugs block TNF-α and decrease the inflammatory activity associated with inflammatory diseases (Tracey, et al., 2008).

2. 3. 5. 2. 2. Cancers

In recent years, the link between cytokine-mediated immunity, inflammation, and cancer has been the focus of considerable attention. A central mediator in this relationship is TNF-α via its role in NF-κB regulation (Tracey, et al., 2008). Indeed, TNF-α-induced activation of NF-κB has been shown to induce the expression of genes that inhibit apoptosis, stimulate cell proliferation, and participate in tumor invasion and metastasis (Tracey, et al., 2008). In liver hepatocytes, Pikarsky and colleagues (2004) have shown that TNF-α produced by neighboring inflammatory stromal cells, activates NF-κB in the hepatocytes, in this manner promoting malignant transformation.

Although genetic inheritance influences the risk of cancer, most of the variation in cancer risk across populations and among individuals is as a result of lifestyle and environmental factors (Longo and Fontana, 2009).

2. 3. 5. 2. 3. Atherosclerosis and dyslipidemia

During the recent years it has become apparent that inflammation plays a key role in the development of atherosclerosis and its clinical manifestations (Lind, 2003). Indeed, IL-6 decreases lipoprotein lipase (LPL) activity and monomeric LPL levels in plasma, which increases macrophage uptake of lipids (Coppack, 2001). In fatty streaks and in the atheromatous ‘cap’ and ‘shoulder’ regions, macrophage foam cells and smooth muscle cells express IL-6, suggesting a role for this cytokine along with IL-1 and TNF-α, in the progression of atherosclerosis (Yudkin, et al., 2000). Beside inhibitory effect on LPL activity, recent study reported that TNF-α activates expression of genes involved in cholesterol synthesis and inhibits expression of bile acid synthesis genes. Moreover, TNF-α was found to decrease HDL level and increase LDL, TG, and TC levels in animal models (Fon Tracer, et al., 2007). In addition, another report has shown that elevated concentrations of IL-6 predict total and cardiovascular mortality over a five year follow-up (Harris, et al., 1999).

2. 3. 5. 2. 4. Noninsulin-dependent diabetes mellitus (NIDDM)

It is well established that subclinical activation of the immune system is involved in the pathogenesis of NIDDM. There is a close relationship between circulating concentrations of CRP, IL-6 and TNF-α, with the components of the insulin resistance syndrome, high TG and low HDL-cholesterol concentrations, and elevated blood pressure (Yudkin, et al., 1999). In fact, over expression of TNF-α in adipose tissue of obese subjects is associated with reduced activity of the insulin receptor, perhaps consequent upon abnormal

phosphorylation of insulin receptor substrate-1 and of the insulin receptor itself (Hotamisligil, et al., 1996). Whereas IL-6 has been found to inhibit glycogen synthesis consequent upon insulin stimulation of isolated hepatocytes (Kanemaki, et al., 1998).

Results from the POSTDAM study (2003) showed that individuals with a combined elevation of IL-6 and IL-1β, rather than the isolated elevation of IL-6, had a roughly threefold increased risk of developing type II DM (Spranger, et al., 2003). Furthermore, IL-6 levels were found increased in diabetic patients and in individuals with metabolic syndrome. These findings suggest a pathogenic role of IL-6 in the development of type II DM (Muller, et al., 2002). In addition, high plasma concentrations of TNF-α were also shown to predict insulin resistence with advancing age (Paolisso, 1998).

Several cytokines cause cardiomyocyte hypertrophy *in vitro*, and they are elevated in pressure overload states leading to the development of cardiac fibrosis and diastolic dysfunction *in vivo*. Indeed, an increased vessel wall stretch and elevated levels of angiotensin II have been shown to activate arterial NF-$_k$B (Hernandez-Presa, et al, 1997). Furthermore, the inhibition of NF-$_k$B reduces myocardial hypertrophy in response to chronic infusion of angiotensin II (Sciarretta, et al., 2007).

Finally, proinflammatory cytokines are believed to play a pathogenetic role in age-related diseases such as Alzheimer's disease, Parkinson's disease, sarcopenia, and osteoporosis (Bruunsgaard, et al., 2001) (Figure 2.2). In addition, proinflammatory cytokines such as IL-6 are one critical group of proteins that contribute to the bone-wasting effects associated with estrogen deficiency (Scheidt-Nave, et al., 2001).

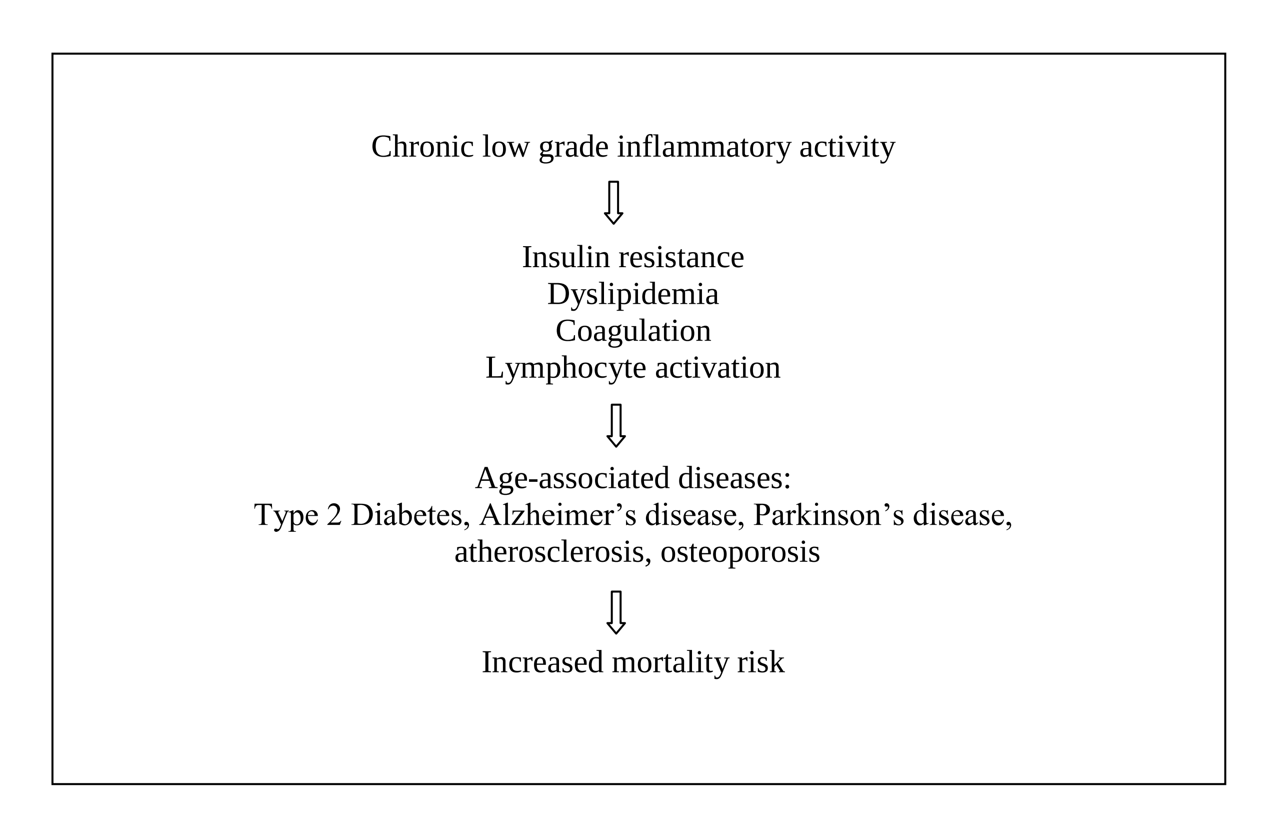


Figure 2.2. Effects of Chronic Low-Grade Inflammation in Age-Associated Diseases (From Bruunsgaard, et al., 2001).

2. 3. 5. 3. Factors affecting circulating proinflammatory cytokine levels

2. 3. 5. 3. 1. Age

Aging is associated with complex changes in most parts of the immune system. The activation and propagation of inflammation in older adults involves multiple soluble mediators that include cytokines, chemokines, CRP, and other inflammatory factors (Pacifici, 1999; O'Mahony, et al., 1998; Bruunsgaard, et al., 2000; 2001). A number of proinflammatory cytokines, including TNF-α, IL-6, and cytokine antagonists, and acute phase proteins are found in increased levels, generally two to fourfold, in the serum of elderly humans and mice, and there is evidence that these higher levels of inflammatory cytokines are linked to increased mortality (Bruunsgaard and Pedersen, 2003). In fact, the age-associated decline in testosterone and estrogen may play mechanistic roles (Crossley and Peterson, 1996).

Interestingly, Sack and colleagues (1998) have investigated the age-dependent levels of these proinflammatory cytokines and other immunological mediators in sera of healthy children (3 to 17 years). They found that IL-1ra, IL-6 and TNF-α showed age-related profiles. Indeed, IL-6 peaked around 3 to 4 years of age as well as at 15 years, whereas, TNF-α showed a clear peak at age of 13 to 14, with a rather sharp fall to adult levels thereafter. In addition, the pattern of IL-1ra consisted of an early peak around 3 years of age and a second peak around age 12, and after 14 years of age, the values were comparable to those of adults. They postulated that IL-1β and IL-6 may play an important physiological role in childhood. Interestingly, this age dependency seems to closely match the physical growth rates and bone formation since IL-1 mRNA and IL-6 mRNA were found in developing bone and cartilage (Sack, et al., 1998).

2. 3. 5. 3. 2. Drugs

Both steroidal and non steroidal anti-inflammatory drugs decrease the circulating proinflammatory cytokine levels, mainly by inhibiting the NF-$_{K}$B pathway (Roman-Blas and Jimenez, 2006). In addition, circulating levels of IL-6 and TNF-α have been shown to be reduced by treatment with statins (Rosenson, et al., 1999). Also both the levels of CRP and IL-6 have been shown to be reduced during aspirin treatment in patients with coronary heart disease (CHD) (Ikonomidis, et al., 1999, Asanuma, et al., 2008).

2. 3. 5. 3. 3. Circulating homocysteine levels

Homocysteine is a marker of cardiovascular risk that may have inflammatory effects (Asanuma, et al., 2008). High concentrations of homocysteine activate endothelial cells and leukocytes, converting them to proinflammatory phenotypes (Poddar, et al., 2001). Activated endothelial cells have shown to up-regulate adhesion molecules and release various cytokines including IL-1, IL-6 and TNF-α. (Dalal, 2003). Nutritional deficiencies in the vitamin cofactors (folate, vitamin B_{12}, and vitamin B_6) required for homocysteine catabolism may promote hyperhomocysteinemia (Kang, et al., 1987, Stabler, et al., 1988). Chronic moderate hyperhomocysteinemia in healthy subjects may have activating effects on immune system regulators like IL-6 (Aksungar, et al., 2007).

2. 3. 5. 3. 4. Circulating leptin levels

Leptin is a 16-kDa weight, cytokine-like hormone synthesized mainly but not exclusively by white adipose tissue (Ahima and Flier, 2000). Leptin acts as a negative feedback signal for neurons in the central nervous system to decrease food intake, and to increase energy expenditure (Ahima and Flier, 2000). Alternatively, leptin plays an important role in modulating inflammatory response and autoimmune reactivity (Fantuzzi and Faggioni, 2000). In fact, leptin exerts proliferative and antiapoptotic activities on a

variety of cell types such as T lymphocytes (Fantuzzi and Faggioni, 2000). It also affects cytokine production, the activation of monocytes/macrophages, wound healing, angiogenesis, and hematopoiesis (Fantuzzi and Faggioni, 2000). In addition, leptin shows anti-inflammatory effects by inducing IL-1ra secretion (Seven, et al., 2009).

2. 3. 5. 3. 5. Genetic polymorphism

Research has identified a polymorphism in the promoter region of the IL-6 gene, that is a G:C change at position -174. *In vitro* expression studies have shown that the C allele is a slightly less efficient promoter than the G allele, suggesting that individuals homozygous for the C allele may have decreased IL-6 concentration (Fishman, et al., 1998).

In addition, mutations analysis has identified a G:A transition in the promoter region of TNF-α gene at position -308. This polymorphic variant has been shown to affect the promoter region of the TNF-α gene leading to a higher rate of transcription compared the wild allele (Wilson, et al., 1997). Accordingly, G308A genotype is associated with higher TNF-α levels than G308G genotype (Aller, et al., 2010).

Finally, two linked IL-1β single nucleotide polymorphisms that increase IL-1β expression (-511 C to T) and (-31 T to C) were identified (Kamangar, et al., 2006).

2. 3. 5. 3. 6. Physical activity

It is well recognized that regular exercise offers protection against all-cause mortality, primarily those associated with chronic low-grade systemic inflammation such as type 2 DM and CHD. Cytokines like IL-6 may be involved in mediating the health-beneficial effects of exercise (Petersen and Pedersen, 2005). Indeed, during exercise, IL-6 is produced and released by contracting skeletal muscle fibers. Consequently, circulating levels of IL-6 significantly increase (up to 100-fold) with exercise and decline in the post exercise period (Hursting, et al., 2006). Interleukin-6 may alternatively inhibit the production of the

proinflammatory cytokines, like TNF-α and IL-1β, and induce the production of the anti-inflammatory mediators like IL-10 and IL-1ra (Tilg, et al., 1994). Hence, this exercise-induced increase in IL-6 may contribute to a reduction of chronic inflammation by reducing proinflammatory mediators (IL-1β and TNF-α) and elevating anti-inflammatory mediators (IL-10 and IL-1ra) (Hursting, et al., 2006).

2. 3. 5. 3. 7. Body weight and composition

Adipose tissue is now recognized as an active endocrine organ that secrets numerous adipokines and cytokines, including leptin, adiponectin, TNF-α, IL-6, and IL-1β (Figure 2.3) (Federico, et al., 2010). In addition, obesity is associated with a low-grade inflammation due to the infiltrated and activated macrophages in white adipose tissue of obese individuals (Federico, et al., 2010). These adipose tissue macrophages are responsible for most of the cytokine production, in particular IL-6 and TNF-α (Weisberg, et al., 2003). In fact, It has been estimated that as much as a third of total circulating concentrations of IL-6 originate from adipose tissue (Mohamed-Ali, et al., 1997). In addition, both *in vitro* and *in vivo* studies suggest a greater contribution from visceral than subcutaneous (SQ) fat in this regard (Fried, et al., 1998; Fontana, et al., 2007). Interestingly, weight loss is associated with a reduction in the macrophage infiltration of white adipose tissue and an improvement of the inflammatory profile of gene expression (Bastard, et al., 2006). Accordingly, moderate weight loss significantly lowered TNF-α and IL-6 concentrations (Corpeleijn, et al., 2005).

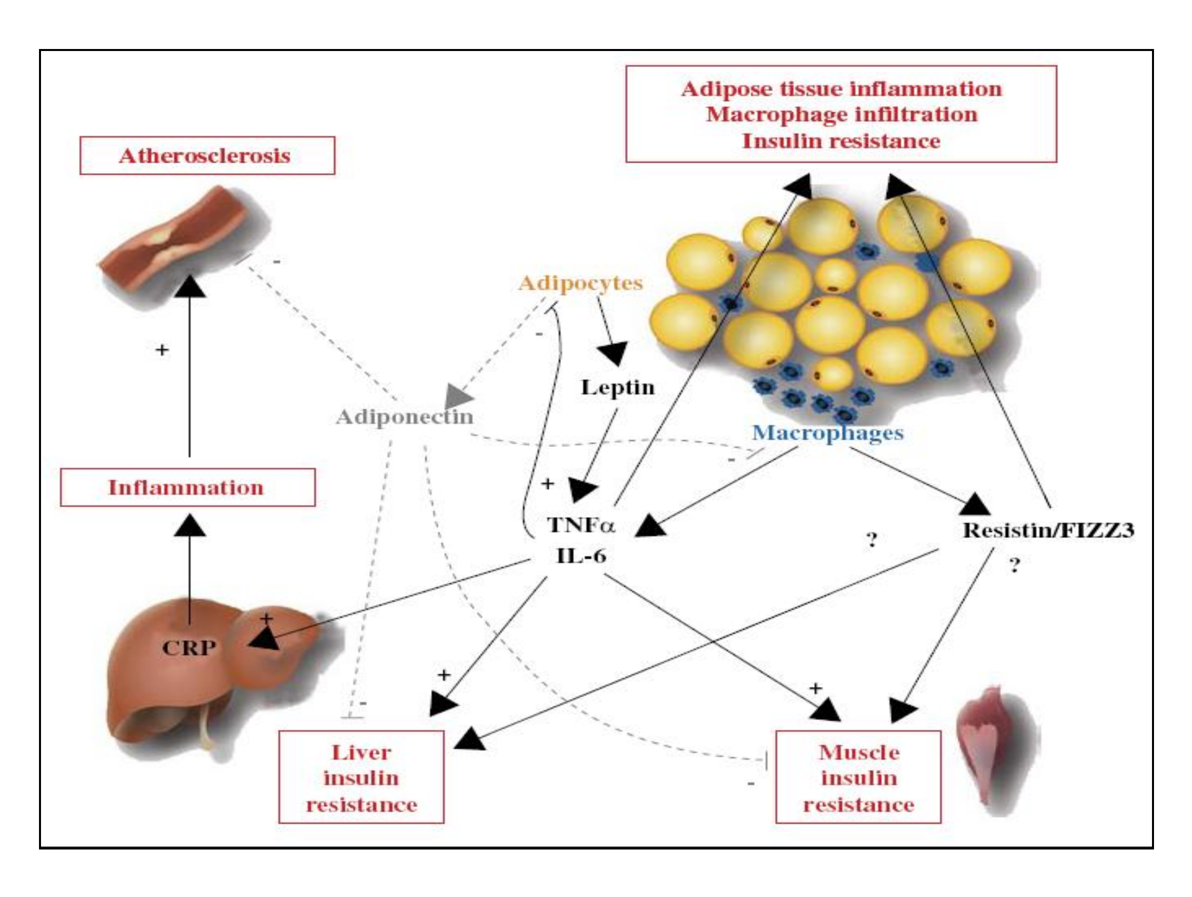


Figure 2.3. Cytokine Expression and Secretion by Adipose Tissue in Obese Subjects (From Bastard, et al., 2006)

2. 3. 5. 3. 8. Nutrition and anti-oxidant nutrients

Diet has a major role in modulating the risk of development of several diseases. Postprandial hyperglycemia acutely increases circulating concentrations of IL-6 and TNF-α by an oxidative mechanism (Esposito, et al., 2002). In addition, high-fat meal increases the plasma levels of TNF-α, and IL-6 (Nappo, et al., 2002, Blackburn, et al., 2006). Corpeleijn and colleagues (2005) suggested that high-fat meal may be an important metabolic stressor, evoking IL-6 release from skeletal muscle. Interestingly, increased postprandial TNF-α and IL-6 levels in healthy subjects could be partly explained by the elevation of the number of monocytes expressing TNF-α within adipose tissue after a fat meal (Hyson, et al., 2002).

Foods high in antioxidants are powerful inflammation fighters. Fruits and vegetables are the best source of dietary antioxidants such as vitamin C, vitamin E, and β-carotene (Holt, et al., 2009). Curcumin, derived from the rhizome of turmeric (*Curcuma longa),* was found to inhibit the activity of the transcription factor NF-$_k$B, which activates the expression of many proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β (Sikora, et al., 2010). In addition, grape flavonoids decreased TNF-α and IL-6 concentrations in both pre- and postmenopausal women (Zern, et al., 2005). Holt and colleagues (2009) investigated the effect of diet high in fruit and vegetables on inflammatory markers. Interestingly, serum IL-6 was inversely associated with intakes of legumes, vegetables, β-carotene, and vitamin C, whereas, serum TNF-α was inversely associated with β-carotene and luteolin.

There is an accumulating evidence supporting the anti-inflammatory effect of IF and CR (Johnson, et al., 2007; Meyer, et al., 2006; Holloszy and Fontana, 2007). In addition, some evidence arising from few studies suggests RIF to have some positive effects on circulating levels of IL-6 and TNF-α. Indeed, IL-6, IL-8, and TNF-α levels were

significantly decreased during RIF (Aksungar, et al., 2007; Unalacak, et al., 2011). However, there is no available evidence regarding effect of RIF on circulating IL-1β levels.

2. 4. Intermittent Fasting

Two different dietary restriction regimens have proven beneficial effects in both animal and human studies, including CR and IF. Caloric restriction is defined as “a reduction in energy intake without malnutrition”, whereas IF, also known as alternate day fasting (ADF), involves a “feast day” on which food is consumed *ad libitum* (ad) that alternates with a “fast” day on which food is withheld or reduced. The fast and feast periods are typically 24 h each, but they may vary. Interestingly, overall caloric intake need not to be limited in intermittent fasting, instead, the frequency of food consumption is altered (Varady and Hellerstein, 2007).

While research on dietary restriction (both IF and CR) in humans is still at an early stage, a large body of evidence for the physiological benefits and life-extending properties of dietary restriction emerges from animal studies (Mattson and Wan, 2005; Varady and Hellerstein, 2007; Fontana, 2009; Wan, et al., 2010). Furthermore, these physiological benefits were maintained by reducing meal frequency without reducing caloric intake (Nakamura. et al., 2004; Anson, et al., 2003; Mattson and Wan, 2005).

Two mechanisms are believed to be responsible for health extending effects of intermittent fasting; a decreased oxyradical production and increased cellular stress resistence (Mattson and Wan, 2005; Martin, et al., 2006). The first mechanism is based on the fact that the majority of oxyradicals in cells are produced in mitochondria during the process of energy metabolism. Because less glucose is available during intermittent fasting, fewer oxyradicals are produced over time, and so there is less free radical-mediated damage to proteins, DNA, and membrane lipids. The second mechanism is based on the fact that the

mild metabolic stress associated with IF induces cells to produce proteins that increase cellular resistance to disease processes, such as heat-chock proteins and brain-derived neutrophic factor (BDNF) (Duan, et al., 2001).

2. 4. 1. Health-related benefits of intermittent fasting

Results from experimental and epidemiologic studies suggest IF to have numerous health benefits effectively reducing morbidity and mortality (Mattson and Wan, 2005; Varady and Hellersetein, 2007; Castello, et al., 2009; Wan, et al., 2010).

2. 4. 1. 1. Effect on CVD risk factors

In animal studies, the ADF was as beneficial as CR in reducing heart rate and both systolic and diastolic blood pressures. Alternate day fasting causes decreased activity of the sympathetic nervous system and this may play an important role in the ability of ADF to decrease blood pressure (Mattson and Wan, 2005). Also noted was a reduction in circulating lipids; TC and TG (Mattson and Wan, 2005). On the other hand, Heilbronn and colleagues (2005) have examined the effect of ADF on CVD risk in healthy volunteers. They found an increase in circulating HDL cholesterol and a decrease in TG concentrations.

Diabetes is one of the major risk factors for cardiovascular diseases. The risk for type 2 DM is greatly increased by excessive energy intakes and the dietary restriction patterns contribute to an improvement in metabolic status of type 2 DM. In animal models, FBG and insulin concentrations have generally been reported to decrease in response to IF (Argentino, et al., 2005; Belkacemi, et al., 2011). However, evidence from human trials suggests that IF does not alter concentrations of glucose but may beneficially modulate other indexes of diabetes risk, such as insulin sensitivity and glucose tolerance (Heilbronn, et al., 2005; Holloszy and Fontana, 2007; Halberg, et al., 2005). Accordingly, it was

postulated that humans may need to fast for longer than 12 h for the reduction in fasting glucose concentrations to be observed (Heilbronn, et al., 2005).

A recent study by Wan, *et al.* (2010) investigated the cardioprotective effect of IF in rats. They found that IF resulted in increased levels of circulating adiponectin known to have cardioprotective and anti-inflammatory actions. Likewise, human plasma adiponectin was increased after two weeks of IF (Halberg, et al., 2005). Interestingly, fasting one day per month practiced by Latter-day Saints population resulted in a lower CHD risk among diabetic patients (Horne, et al., 2008).

2. 4. 1. 2. Effect on cancer risk

The recent shift from traditional dietary patterns to Western diet patterns in many countries has produced a striking increase in the most common cancers. Sine CR reduces cancer incidence by 50 % in monkeys (Longo and Fontana, 2009), and since ADF promotes cancer survival in rats (Siegel, et al., 1988), it is worth to investigate the effect of IF on human cancers. However, to the best of our knowledge, the direct effect of ADF on cancer has been tested only in animal models. Most of these animal studies found a decrease in lymphoma incidence, longer survival after tumor inoculation, and lower rates of proliferation of several cell types (Varady and Hellerstein, 2007). Similarly, studies have linked IF with a consistent reduction in circulating levels of growth factors, anabolic hormones, inflammatory cytokines and oxidative stress markers associated with various malignancies (Longo and Fontana, 2009). Therefore, it is suspected that IF would prevent the initiation, promotion, and progression of many human cancers through a series of metabolic, molecular and cellular adaptations.

2. 4. 1. 3. Effect on lifespan

Experiments performed during the last years showed that IF increases the lifespan of rodents and monkeys. Both mean and maximum life spans are increased by IF in different animal species (Mattson and Wan, 2005). Most probably, this effect may be due to health promotion. However, the exact mechanism of this lifespan extension is still unclear.

2. 4. 1. 4. Effect on inflammatory biomarkers

Human studies investigating the anti-inflammatory effect of IF are scarce and controversial. Halberg and colleagues (2005) did not find out any effect of IF on circulating TNF-α and IL-6 levels in healthy men, whereas circulating levels of TNF-α were significantly decreased by ADF in overweight adults with moderate asthma (Johnson, et al., 2007). In addition, serum TNF-α levels were found to be reduced in humans on long-term CR, with values approximately 50% lower than those of age-matched controls (Meyer, et al., 2006).

On the other hand, animal models of IF have shown decreased circulating concentrations of IL-6, TNF-α, and IL-1 (Castello, et al., 2010), and CR down-regulated the circulating concentrations of TNF-α and IL-6 in diabetic rats (Ugochukwu and Figgers, 2006; 2007). Interestingly, it has been shown that IF has a protective effect on $NF\text{-}_{k}B$ activation (Castello, et al., 2010). This protective effect is of particular interest in view of $NF\text{-}_{k}B$'s involvement in the transcription of proinflammatory genes (Nishikori, 2005).

It is well established that excessive food intake generates oxidative stress, which in turn, stimulates pro-inflammatory mediators (Schmidt, et al., 2005). The protective effect of IF against oxidative stress may be partly explained by decreased reactive oxygen species (ROS) during energy metabolism (Descamps, et al., 2005). In addition, the transcription factor $NF\text{-}_{K}B$ is known to be redox sensitive (Portugal, et al., 2007). Thus, by protecting

against oxidative stress (Descamps, et al., 2005; Lee, et al., 2006), IF down-modulates NF-$_{k}$B activation.

Luna-Moreno and colleagues (2009) have shown that IF modified the 24 h rhythmic variations of pro-inflammatory cytokines. In fact, in the experimental group fed by IF pattern, no differences between dark and light periods were observed in the levels of IL-1 and IL-6, whereas the control group fed *al* showed increased levels of both cytokines during the light period.

Of particular interest is that experimental studies have also shown that both chronic CR and IF may prevent autoimmune diseases (Kubo, et al., 1992; Nakamura, et al., 2004), and attenuate various inflammatory reactions such as ulcerative dermatitis (Klebanov, et al., 1995; Perkins, et al., 1998; Dong, et al., 2000; Nakamura, et al., 2004). This attenuation was associated with decreased expression and secretion of proinflammatory cytokines (Dong, et al., 2000). On the other hand, caloric restriction reduced inflammatory symptoms of patients with atopic dermatitis (Kouda, et al., 2000).

Several mechanisms were proposed to explain the suppressive effect of IF on allergic inflammation, including hypercorticism and reduced oxidative stress during fasting period (Nakamura, et al., 2004). Decreased leptin levels (Ahima, et al., 1996) may also account for this anti-inflammatory effect of IF, since the immunosuppression associated with acute starvation may be reversed by leptin administration (Hursting, et al., 2006).

2. 4. 2. Anti-inflammatory mechanisms of intermittent fasting

Many of the effects of IF are likely mediated by regulation of gene expression, with (1) up-regulation of genes involved in protection against oxidative damage, and (2) down-regulation of genes involved in mediating inflammation (Fontana, 2009). In addition, several metabolic adaptations contribute to the IF-mediated effects, including reduced free

radical-induced tissue damage, improved insulin sensitivity, neuroendocrine adaptive responses, and decreased inflammation (Fontana and Klein, 2007).

Intermittent fasting may suppress inflammatory processes by several pathways, including (1) reduced adiposity and circulating adipokines (Ahima, et al., 1996; Federico, et al., 2010) and cytokines (Corpeleijn, et al., 2005); (2) reduced oxidative stress (Descamps, et al., 2005; Lee, et al., 2006); (3) reduced glycemia (Belkacemi, et al., 2011), since hyperglycemia has been shown to up-regulate the *in vitro* secretion of inflammatory cytokines (Morohoshi, et al., 1996); (4) reduced postprandial monocyte activation (Motton, et al., 2007), since postprandial lipemia subsequent to a fat-containing meal was associated with increased percentages of monocytes expressing intracellular cytokines, including IL-1β and TNF-α (Hyson, et al., 2002), and since meal frequency is reduced during intermittent fasting; and (5) increased HDL levels (Heilbronn, et al., 2005), as emerging evidence indicates that HDL has anti-inflammatory (Navab, et al., 2005; Spieker, et al., 2004) and anti-oxidant properties (Podrez, 2010). In addition, HDL inhibits the ability of antigen-presenting cells to stimulate T cells (Yu, et al., 2010), and limits the expression of proinflammatory cytokines (Spieker, et al., 2004).

2. 5. Ramadan Intermittent Fasting (RIF)

Different populations practice IF worldwide usually as part of their religious rituals. Ramadan occurs in the ninth month of the lunar calendar, lasting between 29 to 30 days. The lunar calendar does not correspond to the Gregorian calendar; therefore Ramadan's occurrence can vary from one season to another (Latifynia, et al., 2007). During this month, adult healthy Muslims refrain from eating, drinking, smoking, or having sexual relationships from down to sunset. This fasting is complete, intermittent, and does not necessarily imply decreased caloric intake (Roky, et al., 2004). The duration of restricted

food and beverage intakes ranges between 12-17 h, according to the solar calendar. Thus, Ramadan constitutes a unique model of prolonged intermittent fasting (Aksungar, et al., 2007).

2. 5. 1. Dietary patterns during Ramadan

Within the same population, large variation in food habits was observed during this religious fasting of Ramadan compared with the rest of the year (El Ati, et al., 1995). First, the energy intakes and hydration are displaced to night hours. Then, the meal frequency is reduced to one major meal after sunset, and another small meal just before down. In addition, the quality and quantity of ingested nutrients change during this month (Roky, et al., 2004). The proportion of fat, protein, and carbohydrate intakes can differ during Ramadan period comparing to the non fasting periods (Adlouni, et al., 1998). Furthermore, there is paramount variability in food habits tendency among different populations practicing Ramadan. Indeed, an increase in protein and lipid intakes was observed in Tunisian population (Roky, et al., 2004; Maughan, et al., 2008), whereas, an increase in total energy intakes was observed in Moroccan population during this month, and was mainly based on carbohydrates (Adlouni, et al., 1998). In addition, changes in ingested fat towards increased mono and poly unsaturated fatty acids have been observed in fasting individuals (Adlouni, et al., 1998).

2. 5. 2. Physiological changes associated with RIF

Long lasting modifications in the circadian distribution of eating and sleeping schedule, together with decreased physical activity during Ramadan, result in several metabolic adaptations (Al Hourani, et al., 2009). It has been suggested that these metabolic adaptations start at the end of the first week (Lamri-Senhadji, et al., 2009). During the past forty years, several studies were undertaken to elucidate the physiological and

psychological changes of RIF in both healthy and patient subjects. Results of these studies are summarized in tables 2.1-2.5. However, these results showed discrepancies, which could have several explanations. The most important one is the difference in protocols followed. The non-fasting control, choice of the experiment day during Ramadan, and the timing of blood sampling were not always comparable (Roky, et al., 2004). In addition, within the same study, the diurnal variation of some parameters (e.g. leptin) during Ramadan (Schoeller, et al., 1997), may lead to erroneous conclusions, even if the sampling is conducted at the same time-points during fasting and non fasting periods. Other explanations could be related to the difference in nutritional customs and habits as well as the climate specificities and the seasonal occurrence of the Ramadan month (Roky, et al., 2004). The discrepancy might also be attributed to ethnic and genetic background of the studied populations (Al Hourani, et al., 2009). Nowadays, in spite of the rising number of studies investigating health-related benefits of Ramadan fasting, the exact mechanisms mediating these physiological changes remain unknown.

2. 5. 2.1. Effect of RIF on CVD risk factors

Some studies found a significant decrease in both systolic and diastolic blood pressures in healthy volunteers (Table 2.1), most probably due to the concomitant decrease in body weight given the reported association between body mass index (BMI) and both systolic and diastolic blood pressures (Dewanti, et al., 2006; Mansi, 2007). However, in clinical studies, the blood pressure in treated hypertensive patients did not decrease during RIF (Perk, et al., 2001). Al Suwaidi and colleagues (2004) demonstrated that no significant difference was found in the number of hospitalization for congestive heart failure while fasting in Ramadan when compared to the non-fasting months.

Coagulation variables are also decreased during RIF. In fact, decreased platelet count (Ramadan, et al., 1999), fibrinogen level, and factor VII activity (Sarraf-Zadegan, et al., 2000), along with the increased HDL levels (Mansi, 2007), suggest a beneficial effect on CHD patients. As a matter of fact, Temizhan and colleagues (1999) have evaluated the effect of RIF on a population of CHD patients. They found that the number of cases with acute CHD events was lower in Ramadan, thus confirming the beneficial effect of RIF in such population.

Table 2.1. Changes in Blood Pressure and Coagulation Variables during RIF.

Reference	**Sex & No. of subjects**	**SBP**	**DBP**	**Platelets**	**Fibrinogen**	**Factor IIV**	***D*-Dimer**
Furuncuoglu, et al., (2007).	32F & 7M			↓			
Aybak, et al., (1996).	20M			↓			
Sarraf-Zadegan, et al., (2000).	22M & 28F			↑*	↓*	↓*	
Ramadan, et al., (1999).	13M			↓*			
Shariatpanahi, et al., (2008).	55M	↓*	↓*				
Aksungar, et al., (2005).	12M & 12F				↑		↓*
Mansi, (2007).	70M & F	↓*	↓*				
Dewanti, et al., (2006).	37M	↓*	↓*				
Perk, et al., (2001).	15M & 2F	↓	↓				
Bernieh, et al., (2010).	19M & 12F	↓	↓				

*Statistically significant; M: male, F: female; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Considering lipid metabolism, a large body of evidence showed a beneficial effect of RIF (Table 2.2). High density lipoprotein concentrations significantly increased during RIF (Aksungar, et al., 2005; 2007; Lamri-Senhadji, et al., 2009; Mansi, 2007), while TC, TG, and LDL concentrations were either decreased (Mansi, 2007; Furuncuoglu, et al., 2007), increased (Aybak, et al., 1996; Ramadan, et al., 1999), or remained unchanged (Aksungar, et al., 2005; 2007). In addition, Apo B, the major protein component of LDL, was significantly decreased (Adlouni, et al., 1998; Sarraf-Zadegan, et al., 2000), whereas, Apo A-I, the major protein component of HDL, was significantly increased in fasting subjects (Maislos, et al., 1993; Adlouni, et al., 1998), and remained significantly elevated one month after Ramadan (Adlouni, et al., 1998). Thus, we can postulate that hyperlipidemic patients could benefit from this unique model of IF. However, when assessing the effects of RIF on lipid metabolism in a population of hyperlipidemic patients, Akanji and colleagues (2000) found that Apo A-I and its ratio to Apo B and HDL, significantly increased, whereas, the other lipid parameters (TC, TG, LDL, and HDL) showed no significant change in fasting subjects. Since HDL cholesterol was found to have either anti-inflammatory or proinflammatory properties according to the inflammatory status of the body (Navab, et al., 2005), it was postulated that it is not only the level of HDL that is relevant for its atheroprotective properties. Indeed, the quality of the HDL is also important (Van Lenten, et al., 1995). Proinflammatory HDL composition is marked by its enrichment in SAA, and its poorness in Apo A-I. Whereas, anti-inflammatory HDL composition is marked by its enrichment in Apo A-I (Hu, et al., 2008; Spieker, et al., 2004). As a result, RIF might favorably influence CHD risk in hyperlipidemic subjects by increasing anti-inflammatory HDL.

Table 2.2. Changes in Blood Lipids and Lipoproteins during RIF.

Reference	Sex & No. of subjects	TC	TG	LDL	HDL	Apo A-I	Apo B
Adlouni, et al., (1998).	32M					↑*	↓*
Lamri-Senhadji, (2009).	22M 24F			↓*	↑*		
Saleh, et al., (2005).	41M & 19F	↓	↑	↓*	↑		
Maislos, et al., (1992).	16M & 8F	↓	↑	↑	↑*		
Al Hourani, et al., (2009).	57F	↓	↓*	↑	↑*		
Aksungar, et al., (2007).	20M & 20F	↓	↑	↑	↑*		
Furuncuoglu, et al., (2007).	32F & 7M	↓*	↑*		↓		
Aybak, et al., (1996).	20M		↑*	↓			
Argani, et al., (2003).	15M & 15F	↓		↓	↑*		
Sarraf-Zadegan, et al., (2000).	22M & 28F	↓	↓	↑	↓	↑	↓*
Fedail, et al., (1982).	20M & 4F	↑*	↓				
Chaouachi, et al., (2007).	15M	↑	↓*	↑*	↑*	↑*	↓
Fakhrzadeh, et al., (2003).	50M 41F	↓*	↓*	↓*	↑*		
Shariatpanahi, et al., (2008).	55M				↑*		
El Ati, et al., (1995).	16F	↑	↓				
Chennaoui, et al., (2009).	8M	↓	↓	↓	↑*	↑	↓
Aksungar, et al., (2005).	12M & 12F	↑	↑	↓	↑*		
Ziaee, et al., (2006).	41M 39F	↑	↑	↑	↓*		
Mansi, (2007).	70M & F	↓*	↓*	↓*	↑*		
Akanji, et al., (2000).	33M 31F	↓	↓	↑	↓	↑*	↓*

*Statistically significant; M: male, F: female; TC: total cholesterol; TG: triglycerides; HDL: low density lipoprotein; LDL: low density lipoprotein; Apo A-1: apolipoprotein A-I, Apo B: apolipoprotein B.

Most studies showed a significant decrease in body weight during and at the end of RIF (Table 2.3). Indeed, the change in body weight is mainly due to changes in energy intakes, water balance, and energy expenditure (Hussain and Leeds, 1996). Significant weight loss occurred independently of total energy intakes during RIF (Adlouni, et al., 1998; Al-Hourani and Atoum, 2007; Chaouachi, et al., 2007). In addition, Ramadan and colleagues (1999) found that energy balance was well maintained during RIF. Thus, it has been postulated that weight loss observed during RIF could be attributed to the negative fluid balance (Mustafa, et al., 1978), as body weight often returned to its basal value after Ramadan (Gumaa, et al., 1978; Sweileh, et al., 1992). It is noteworthy that the extent of total body water loss during RIF depends on the fasting season (Ramadan, et al., 1999).

Table 2.3. Changes in Body Weight during RIF.

Reference	Sex & No. of subjects	Mean baseline body weight (Kg)	Mean change in body weight (Kg) [c]	Mean change in energy intake (%)
Lamri-Senhadji, et al., (2009).	24F 22M	53 70	-1.00 0.00	+14.52* +11.30*
Al-Hourani and Atoum, (2007).	57F	57.5	-0.6*	-6.47
Adlouni, et al., (1998).	32M	96.61	-1.78*	+16.4*
Mansi, (2007).	70 M & F	76.64	-3.98*[a]	NA
Ziaee, et al., (2006).	4M & 39F	62.4	-1.2*	NA
Al-Numair, (2006).	45M	85.5	-2.3*	-11.16*
Hallak and Nomani, (1988).	16M	66.2	-2.4*[a]	NA [b]
Chennaoui, et al., (2009).	8M	76.1	-1.5	-8.12*
Yucel, et al., (2004).	34 M & F	68.67	68.64	NA
El Ati, et al., (1995).	16F	59.3	+0.6	0.00
Shariatpanahi, et al., (2008).	55M	80.69	-2.4*	-1.86*
Fakhrzadeh, et al., (2003).	50M 41F	64.9 60.7	-1.2* -0.4	-15.66* -28.40*
Dewanti, et al., (2005).	37M	64.5	-1.5*	NA
Chaouachi, et al., (2007).	15M	68.13	-1.23*	+4.6
Fedail, et al., (1982).	20M & 4F	69.5	-1.8*	NA

*Statistically significant; [a] on the fourth week of Ramadan; [b] volunteers were submitted to hypocaloric diets; [c] calculated as mean baseline body weight – mean body weight during Ramadan; F=female, M=male; NA= not available.

The effect of RIF on diabetes control is largely documented (Maislos, et al., 2001; Salti, et al., 2004; Olgun, 2006; Patel, et al., 2007; Ait Saada, et al., 2008; 2010 ; Al Alwan and Al Banyan, 2010). However, study results showed discrepancies which lead to variations in the management of diabetic patients who are willing to fast during this month. On the other hand, studies conducted on healthy volunteers, and investigating the effect of RIF on FBG and insulin concentrations (Table 2.4), showed conflicting results which may be due to the variations in protocols followed, as well as the variations in food habits among different populations, e.g. some populations showed increased carbohydrate intakes during RIF (Lamri-Senhadji, et al., 2009). Moreover, Iraki and colleagues (1997) have found that the circadian patterns of both insulin and glucose were altered during the month of Ramadan, a matter that implies a necessary revision of the protocols comparing between two different circadian times (Argani, et al., 2003; Saleh, et al., 2005; Bernieh, et al., 2010). However, it is valuable to note that significant decrease in glycosylated hemoglobin (HbA1C) (Ait Saada, et al., 2008; 2010) was reported during and after Ramadan, suggesting improved glycemic control in diabetic patients during RIF.

Hyperuricemia is now recognized as a CHD risk factor. Several studies showing increased serum uric acid levels with RIF suggest increased CHD risk (Gumaa, et al., 1978; Fedail, et al., 1982; El Ati, et al., 1995; Al-Numair, 2006). However, this hyperuricemia was always within the reference ranges. In fact, increased serum uric acid levels may be explained by either hypohydration or by increased protein intake during this month (El Ati, et al., 1995). Thus fasting induced hyperuricemia is reversible as long as hypohydration is corrected by well hydration just after breaking the fast. Additionally, patients with hyperuricemic conditions are allowed not to fast during Ramadan according to Islamic rules.

Table 2.4. Changes in Blood Glucose, Insulin, HbA1C, Uric Acid, Vitamin B12, and Folate during RIF.

Reference	Sex & No. of subjects	BG	Insulin	HbA1C	UA	VitB12	Folate
Saleh, et al., (2005).	41M 19F	↓					
Maislos, et al., (1992).	16M & 8F	↓					
Al Hourani, et al., (2009).	57F				↓		
Aksungar, et al., (2007).	20M 20F					↑*	↑*
Furuncuoglu, et al., (2007).	32F & 7M	↓*			↓*		
Aybak, et al., (1996).	20M	↓*					
Argani, et al., (2003).	15M & 15F	↓			↑		
Sarraf-Zadegan, et al., (2000).	22M & 28F	↓					
Ramadan, et al., (1999).	13M				↑		
Chaouachi, et al., (2007).	15M	↑	↑*		↑*		
Fakhrzadeh, et al., (2003).	50M 41F	↓*					
Shariatpanahi, et al., (2008).	55M	↓*	↓*				
El Ati, et al., (1995).	16F	↑*			↑*		
Al-Numair, (2006).	45M	↓*			↑*		
Mansi, (2007).	70M & F	↓*					
Akanji, et al., (2000).	33M 31F				↓		
Ait Saada, et al., (2010).	66F			↓*			

*Statistically significant; F: female, M: male; BG: blood glucose; HbA1C: glycosylated hemoglobin; UA: uric acid; VitB12: vitamin B12.

2. 5. 2. 2. Effect of RIF on inflammatory biomarkers

Few studies have investigated the effect of RIF on inflammatory biomarkers (Table 2.5). Anti-inflammatory effect of RIF was illustrated by the significant decrease in IL-6 (Aksungar, et al., 2007), CRP (Maughan, et al., 2005; Aksungar, et al., 2007), homocysteine (Aksungar, et al., 2005; 2007), IL-8, and TNF-α (Unalacak, et al., 2011), during RIF. Furthermore, RIF significantly decreased the circulating levels of high sensitivity-CRP in a population of newly diagnosed type II DM patients (Hamdy, et al., 2008). Interleukin-6 and CRP levels remain depleted 20 days after Ramadan (Aksungar, et al., 2007), a matter that implies an extended protective effect against inflammation.

The populations studied by Aksungar, et al. (2005, 2007) and Hamdy, et al. (2008) had mild to moderate daily physical activity. However, in a population of elite judo athletes maintaining high training loads during Ramadan, blood CRP levels increased significantly in the middle and at the end of Ramadan, and returned to baseline value post Ramadan. Additionally, homocysteine level was not affected (Chaouachi, et al., 2009). Given the fact that judo fights may lead to tissue trauma and injury, increased CRP levels may be due to tissue damage occurring in judokas, rather than to increased physiological stress during RIF. Interestingly, IL-6 was significantly increased during RIF in a population of middle-distance runners (Chennaoui, et al., 2009). However, exercise-induced increase in IL-6 may have anti-inflammatory effect by reducing proinflammatory and elevating anti-inflammatory mediators (Hursting, et al., 2006).

As stated earlier, it was postulated that the suppressive effect of short-term intermittent fasting on allergic inflammation may involve depressed leptin activity (Nakamura, et al., 2004). In addition, after 12 h of total fasting, leptin was found to follow a steady decline from the baseline values (Kolaczynski, et al., 1996), and it was postulated that one of the

adaptive physiological responses to fasting is a fall in serum leptin (Kolaczynski, et al., 1996). However, Kassab and colleagues (2003) have reported a significant increase in serum leptin concentrations, when comparing levels obtained from a single daily blood sampling during fasting and non fasting periods. Since changing meals pattern was found to produce phase shifts in plasma leptin levels (Schoeller, et al., 1997), and since notable variations in meals pattern occurs during RIF, results obtained by Kassab and colleagues (2003) during fasting and non fasting periods may not be comparable. Indeed, the 24 h mean concentrations are comparable. Even more, RIF did not affect the leptin 24 h mean concentrations in ten healthy volunteers (Bogdan, et al., 2005). It is noteworthy that this low sample size might fail to find out any statistically significant effect, and that future studies with large scale are needed to investigate the effect of RIF on leptin levels.

On the other hand, the anti-inflammatory mediator haptoglobin increased significantly during RIF (Chaouachi, et al., 2009). Haptoglobin is an anti-oxidant (Carter and Worwood, 2007) and anti-inflammatory (Jue, et al., 1983) marker. Its main function is binding and clearing free hemoglobin, and prevents hemoglobin-driven oxidative tissue damage (Carter and Worwood, 2007). Indeed, free hemoglobin can be harmful to the body, as it promotes the accumulation of hydroxyl radicals resulting in oxidative tissue damage (Carter and Worwood, 2007).

Table 2.5. Changes in Blood Inflammatory Biomarkers during RIF.

Reference	Sex & No. of subjects	IL-6	IL-1	TNF-α	CRP	IL-8	Hc	Lep	Hp	MDA
Aksungar, et al., (2007).	20M & 20F	↓*			↓*		↓*			
Chennaoui, et al., 2009.	8M	↑*			↑					
Aksungar, et al., 2005.	12M & 12F						↓*			
Hamdy, et al., 2008.	29M & 23F				↓*					
Chaouachi, et al., 2009.	15M				↑*			↓	↑*	
Maughan, et al., 2008.	78M				↓*					
Unalacak, et al., 2011.	20M			↓*		↓*				
Bogdan, et al., 2005.	10M							↓		
Ibrahim, et al., 2008.	9M & 5F									↓*

Hc: homocysteine; Lep: leptin; Hp: haptoglobin; MDA: malondialdehyde, a marker of oxidative stress; * statistically significant; M: male, F: female.

3. MATERIALS AND METHODS

3. 1. Subjects

This study was carried out in the month of Ramadan of August/September 2009, and the average duration of fasting was 14-15 hours a day. The mean climate temperature and humidity were 26.3°C and 44.6%, and 23.7°C and 55.8% during August and September respectively. There were no special nutritional regimens and recommendations during the whole study. To maintain sample homogeneity, all participants were chosen from the same living community, Rusaifa city, so that the socioeconomic levels were highly similar.

All participants were required to provide a written informed consent (Appendix A) before the study started. A questionnaire (Appendix B) was administered, in which medical history was recorded through a personal interview with the each subject by well-trained personnel. At the beginning of the study, the number of participants was 120, but 20 subjects decided not to undergo the second stage and another 20 subjects decided not to undergo the third stage. So that, the 80 remaining subjects did undergo all of three stages of the study. Subjects not taking any drug or receiving any medical treatment immediately before or during the study were then selected. So, 50 healthy subjects (21 males aged 18-49 years, and 29 females aged 18-51 years), were selected for the investigation of circulating inflammatory cytokines. It is noteworthy that male subjects fasted during the whole month while female subjects did not fast during their menstrual period, as Islamic rules do not permit fasting during the menstrual period. Accordingly, female subjects might miss 5±2 days of fasting.

3. 2. Blood Sampling

Blood samples were collected from all healthy volunteers one week before Ramadan, on the third week of Ramadan, and one month after the end of Ramadan. Each subject served as his own control by comparing before Ramadan values with those during and after Ramadan. At each visit, systolic and diastolic blood pressures were measured with the subject in a seated position after 5 min rest. Blood sampling was conducted in Al-Quds Medical Laboratories center between 10 am and 4 pm each time. The blood samples were collected and the serum was separated within an hour. The blood samples were centrifuged at room temperature for 5 min. at 4000 rpm (Sigma ® 3-18K lab Centrifuge, Germany). Blood samples were directly analyzed for complete blood count cells (CBC) using Auto hematology analyzer (Mindray® BC-3000, China). For cytokine measurements, serum samples were aliquoted, coded, and stored at -80°C until analyzed. Owing to the insufficient volume of serum collected, TNF-α levels were measured in 48 samples, while IL-1β and IL-6 levels were measured in only 40 subjects. In fact, one of the major disadvantages of ELISA technique is that it requires high volumes of serum.

3. 3. Anthropometric Measurements

During each of the three visits, anthropometric measurements (body weight and height, waist and hip circumferences), and body fat % were performed by well-trained personnel. Body fat was measured by Body Fat Analyzer (GIMA®, Italy). Body mass index (BMI) and waist to hip ratio were calculated. BMI was calculated as body weight (in kg) divided by body height (in meters) squared. Volunteers with BMI < $25Kg/m^2$ are considered to have normal weight, and those with BMI ≥ 25 are considered to be overweight (WHO, 2006).

3. 4. Determination of Cytokine Levels

Samples from the same volunteer were measured in the same assay to reduce the effect of interassay variation on serum cytokine levels. All samples have been thawed the same number of times, as the freeze-thawing cycles may decrease the cytokine concentrations by protein degradation. In addition, the different kits were used within the same week. Serum TNF-α, IL-1β, and IL-6 were each measured by a sandwich ELISA using commercial kits (Peprothech, USA, for TNF-α and IL-6, and R & D systems, DuoSet®, USA for IL-1β). Phosphate-buffered saline (PBS), wash buffer, block buffer, and diluent are solutions required for the ELISA development. They were prepared, as described in the data sheet accompanying each kit, a day before the cytokine measurement, and stored at 4°C for up to 1 week. All reagents were brought to room temperature before preparation.

Circulating IL-6, IL-1β, and TNF-α concentrations were measured as described in the data sheet accompanying each kit. Ninety six-well plates were coated with an anti-cytokine (either an anti-TNF-α, anti-IL-6, or anti-IL-1β) monoclonal antibody, and were incubated overnight at room temperature. After washing, the plates were incubated with a Block Buffer for a minimum of 1 h. After washing, standards and serum samples (100µl) were pipetted into the wells, and incubated for 2 hr. After washing off unbound sample cytokine, a polyclonal anti-cytokine (anti-TNF-α, anti-IL-6, or anti- IL-1β) linked to horseradish peroxidase enzyme, was added to the wells. This detection antibody was incubated for 2 hr, and detected using a substrate solution containing 2,2'-Azino-bis-(3-ethylbenzthiozoline-6-ssulfonic acid) (or 1:1 mixture of H_2O_2 and Tetramethylbenzidine in case of IL-1β).

At the same time that serum samples were tested, a series of wells were prepared with the use of known concentrations of recombinant IL-6, TNF-α, or IL-1β standards. Standard

curves of absorbance versus the known IL-6, TNF-α, or IL-1β concentrations in these wells were plotted.

For IL-6 and TNF-α, the absorbance was detected using a microplate reader (Biotek®, USA) set at a wavelength of 405 nm with a wavelength correction reading at 650 nm. In addition, the plates were monitored each 5 min. for color development for approximately 35 min. However, for IL-1β, the reaction was stopped by Stop Solution ($2NH_2SO_4$), then, the absorbance was immediately determined by the same microplate reader set at a wavelength of 450 nm with a wavelength correction reading at 570 nm. All samples and standards were tested either in triplicate in case of IL-6 and TNF-α or in duplicate in case of IL-1β, and mean values were determined, after excluding the odd values.

Finally, the amount of IL-6, TNF-α, or IL-1β in the unknown serum samples were determined using standard curves.

3. 5. Statistical Analysis

The statistical analysis was performed using the statistical package for social sciences (SPSS), version 16.0 (Chicago, IL, USA). Results are expressed as mean ± standard deviation (SD) and a *P*-value of less than 0.05 was considered to be statistically significant. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (IL-1β, SBP, DBP, and Monocytes count) were analyzed by non parametric tests. Three individuals with outliers values for TNF-α concentrations were excluded from TNF-α data analysis as the values are clearly extreme values that do not belong to the general distribution. Data were analyzed using Student's *t* test (either paired or unpaired) and Mann-Whitney and Wilcoxon non parametric tests, by comparing values before and after Ramadan with those during Ramadan. Correlation tests

were performed using the Pearson parametric correlation test for normally distributed variables, and Spearman non parametric test for skewed variables.

4. RESULTS

4. 1. Characteristics of study subjects

Table 4.1. Characteristics of Study Subjects before, during, and after Ramadan, Subdivided According to Sex (n=50, mean age: 32.70±9.46).

Parameter	Before Ramadan			During Ramadan			After Ramadan		
	Male	Female	*P*-value[a]	Male	Female	*P*-value[a]	Male	Female	*P*-value[a]
Inflammatory cytokines									
IL-6 (pg/ml)[b]	146.27± 116.22	161.60± 126.06	0.704	77.53± 71.83	61.72± 34.13	0.432	92.20± 64.53	120.04± 80.79	0.264
IL-1β (pg/ml)[b]	15.55± 18.60	19.23± 17.76	0.538	6.25± 6.44	2.15±2.71	**0.04**	14.50± 19.78	19.44± 15.82	0.390
TNF-α (pg/ml)[c]	184.26± 146.56	176.23± 118.53	0.84	61.05± 75.95	45.79± 38.89	0.38	134.68± 124.36	157.08± 118.40	0.540
Immune cells									
Total leukocytes ($10^9.L^{-1}$)	6.63± 1.48	6.82± 1.83	0.684	6.07± 1.31	5.58±1.48	0.231	6.5± 1.62	6.52± 2.12	0.695
Granulocytes ($10^9.L^{-1}$)	4.01± 1.22	4.03± 1.5	0.969	3.49± 0.98	3.4±1.36	0.801	3.52± 1.19	3.82± 1.41	0.436
Lymphocytes ($10^9.L^{-1}$)	2.23± 0.53	2.38± 0.81	0.473	2.25± 0.6	1.92± 0.41	**0.024**	2.12± 0.72	2.21± 0.97	0.73
Monocytes ($10^9.L^{-1}$)	0.41± 0.16	0.41± 0.14	0.847	0.31± 0.12	0.27± 0.08	0.261	0.75± 0.38	0.43± 0.30	**0.002**
Anthropometric characteristics									
Body weight (kg)	73.27± 13.80	70.79± 13.26	0.53	72.38± 13.93	69.27± 13.23	0.418	73.40± 13.93	70.86± 13.32	0.518
BMI (kg/m^2)	24.15± 4.34	27.85± 4.92	0.009	23.89± 4.18	27.27± 4.98	**0.015**	24.62± 4.55	27.85± 4.92	**0.022**
HC (cm)	98.35± 7.96	105.69± 9.73	0.007	97.66± 8.50	104.97± 9.97	**0.009**	98.19± 8.42	103.07± 10.13	0.078
WC (cm)	85.38± 11.47	82.34± 10.97	0.348	84.33± 11.25	81.50± 9.56	0.344	84.61± 10.52	81.27± 10.22	0.265
Body fat (%)	13.79± 8.29	31.60± 9.50	0.000	11.69± 6.90	26.67± 9.62	**0.000**	20.02± 9.18	38.06± 7.01	**0.000**

[a] When comparing between male and female groups. [b] 15 male, 25 female; [c] 19 male, 26 female; HC: hip circumference, WC: waist circumference, BMI: body mass index; results are expressed as mean±SD; $P \leq 0.05$ is considered to be statistically significant.

4.2. Cytokines and Immune Cells

Circulating IL-1β, IL-6, and TNF-α levels were significantly lower during Ramadan, and IL-6 levels still remained depleted one month after Ramadan when compared to basal (before Ramadan) values (Table 4.2). During Ramadan, TNF-α, IL-6, and IL-1β decreased by 57.10 %, 39.90 %, and 9.97 % respectively (Table 4.3).

Total leukocytes, lymphocytes, granulocytes, and monocytes counts were significantly decreased during Ramadan, and monocytes count was significantly increased after Ramadan (Table 4.2).

Table 4.2. Circulating Levels of Proinflammatory Cytokines and Immune Cells before, during, and after RIF.

Parameter	Before Ramadan	During Ramadan	*P*-value[c]	After Ramadan	*P*-value[c]
Proinflammatory cytokines					
IL-6 (pg/ml)[a]	155.85±121.18	67.42±51.25	**0.000**	109.6±75.6	**0.030**
IL-1β (pg/ml)[a]	17.84±17.92	3.89±4.84	**0.000**	17.59±17.33	0.942
TNF-α (pg/ml)[b]	179.62±129.56	52.22±57.25	**0.000**	147.62±120.07	0.140
Immune cells					
Total leukocytes ($10^9.L^{-1}$)	6.74±1.67	5.79±1.42	**0.000**	6.51±1.91	0.335
Granulocytes ($10^9.L^{-1}$)	4.02±1.37	3.44±1.20	**0.000**	3.70±1.32	0.069
Lymphocytes ($10^9.L^{-1}$)	2.32±0.71	2.06±0.52	**0.006**	2.17±0.86	0.155
Monocytes ($10^9.L^{-1}$)	0.41±0.15	0.29±0.10	**0.000**	0.57±0.37	**0.009**

[a] n=40; [b] n=45; [c] when statistically compared to before Ramadan (basal) levels. Results are expressed as mean ± SD; $P \leq 0.05$ is considered to be statistically significant.

Table 4.3. Percentage of Changes in Cytokine Levels during RIF (mean ± SD).

Cytokine	Mean ±SD (%)
IL-1β	-9.97±19.70
IL-6	-39.90±42.85
TNF-α	-57.10±49.85

4. 3. Anthropometric Measurements

The mean values of body weight, BMI, body fat, and waist and hip circumferences obtained before, during, and after Ramadan, are listed in Table 4.4. Ramadan fasting caused significant decrease in body weight, BMI, and body fat %. The latter measurement was significantly increased after Ramadan. In contrast, both waist and hip circumferences were slightly decreased during Ramadan, and then they were significantly decreased after Ramadan, when compared to basal values.

Table 4.4. Anthropometric Characteristics of Study Subjects before, during, and after RIF (mean ± SD).

Parameter	Before Ramadan	During Ramadan	*P*-value[a]	After Ramadan	*P*-value[a]
Body weight (kg)	71.82±13.41	70.58±13.20	**0.000**	71.92±13.50	0.592
BMI (kg/m^2)	26.30±5.01	25.85±4.91	**0.000**	26.49±4.99	0.374
HC (cm)	102.61±9.66	101.90±9.98	0.093	101.02±9.67	**0.002**
WC (cm)	83.62±11.17	82.69±10.34	0.069	82.68±10.37	**0.047**
W:H ratio	0.81±0.07	0.81±0.07	0.630	0.81±0.06	0.523
Body fat (%)	24.12±12.60	20.38±11.32	**0.000**	30.48±11.32	**0.000**

[a] When statistically compared to before Ramadan (basal) levels; BMI: body mass index; HC: hip circumference; WC: waist circumference; Results are expressed as mean ± SD; $P \leq 0.05$ is considered to be statistically significant.

4. 4. Changes in Blood Pressure before, during, and after RIF.

Both systolic and diastolic blood pressures were significantly decreased during Ramadan, and then returned to basal values one month after Ramadan (Table 4.5).

Table 4.5. Effect of Ramadan fasting on Blood Pressure (mean ± SD).

Blood pressure	Before Ramadan	During Ramadan	*P*-value	After Ramadan	*P*-value
SBP (mmHg)	112.30±10.01	104.40±9.07	**0.000**	111.82±9.60	0.83
DBP (mmHg)	76.20±8.48	71.60±10.40	**0.008**	74.50±14.40	0.422

SBP: Systolic blood pressure; DBP: diastolic blood pressure; Results are expressed as mean ± SD; $P \leq 0.05$ is considered to be statistically significant.

When comparing male to female subjects, BMI, HC, and body fat % were higher in female than in male subjects. Monocytes count was significantly higher in male subjects after Ramadan, and lymphocytes count was significantly higher in male subjects during Ramadan (Table 4.1).

When splitting the data by two age groups, there was no significant difference in circulating cytokine levels between subjects aged 40 years and more, and those aged less than 40 year (Table 4.6). Also there was no significant difference between overweight and normal weight subjects (Table 4.7).

Table 4.6. Circulating Cytokine Levels before, during, and after RIF in Study Subjects Subdivided According to Age (mean±SD).

Cytokine	Before Ramadan			During Ramadan			After Ramadan		
	≥40 (n=9)	<40 (n=31)	*P*-value	≥40 (n=9)	<40 (n=31)	*P*-value	≥40 (n=9)	<40 (n=31)	*P*-value
IL-6 (pg/ml)	197.00± 138.18	143.90± 133.90	0.252	70.44± 35.14	66.55± 55.52	0.855	95.33± 38.37	113.74± 83.28	0.527
IL-1β (pg/ml)	27.49± 25.27	15.04± 14.53	0.235	1.65± 1.66	4.27± 5.30	0.092	13.33± 11.22	18.83± 18.70	0.409
	(n=9)	**(n=36)**		**(n=9)**	**(n=36)**		**(n=9)**	**(n=36)**	
TNF-α (pg/ml)	189.44± 162.61	177.17± 122.59	0.803	28.11± 28.52	58.25± 61.22	0.160	134.89± 140.05	150.81± 116.57	0.726

Results are expressed as mean ± SD; *P*≤0.05 is considered to be statistically significant.

Table 4.7. Circulating Cytokine Levels before, during, and after Ramadan in Study Subjects Subdivided According to BMI (mean±SD).

Cytokine	Before Ramadan			During Ramadan			After Ramadan		
	BMI<25 (n=16)	BMI≥25 (n=24)	*P*-value	BMI<25 (n=18)	BMI≥25 (n=22)	*P*-value	BMI<25 (n=13)	BMI≥25 (n=27)	*P*-value
IL-6 (pg/ml)	153.81± 128.12	157.21± 119.12	0.933	66.61± 59.22	68.50± 45.19	0.910	102.41± 43.62	124.54± 118.48	0.525
IL-1β (pg/ml)	19.21± 17.84	16.94± 18.31	0.699	5.25± 6.20	2.40± 2.94	0.087	22.22± 21.61	15.36± 14.79	0.315
	(n=19)	**(n=26)**		**(n=21)**	**(n=24)**		**(n=17)**	**(n=28)**	
TNF-α (pg/ml)	189.05± 146.00	172.27± 118.58	0.692	53.72± 69.59	44.08± 61.52	0.313	162.12± 112.54	138.82± 125.60	0.534

Results are expressed as mean ± SD; *P*≤0.05 is considered to be statistically significant.

When correlation tests were performed (Tables 4.8 - 4.12), IL-1β levels were positively associated with IL-6 and TNF-α levels before and after Ramadan. Tumor necrosis factor-α levels were positively associated with SBP after Ramadan, and IL-1β and Il-6 levels were positively correlated with DBP after Ramadan. In addition, Strong positive correlation was found between body weight and boy fat percentage during all of the three study time-points (Table 4.13).

Table 4.8. Associations of TNF-α before, during, and after RIF.

Variables	Before Ramadan		During Ramadan		After Ramadan	
	Pearson ’r	***P*-value**	**Pearson ’r**	***P*-value**	**Pearson ’r**	***P*-value**
IL-6	0.113	0.518	0.151	0.386	0.090	0.956
BMI	0.127	0.407	0.256	0.088	0.144	0.346
Body fat%	0.152	0.318	0.201	0.186	0.100	0.514
Total leukocytes	0.248	0.100	0.140	0.360	0.016	0.917
lymphocytes	0.095	0.535	0.046	0.766	0.013	0.932
Granulocytes	0.248	0.103	0.111	0.466	0.095	0.533
Monocytes[a]	0.069	0.651	0.115	0.450	0.078	0.611
Age	0.155	0.309	0.132	0.386	0.090	0.558

[a] Correlation test was performed using Spearman non parametric test; $P \leq 0.05$ is considered to be statistically significant.

Table 4.9. Associations of IL-6 before, during, and after RIF.

Variables	Before Ramadan		During Ramadan		After Ramadan	
	Pearson’s rho	***P*-value**	**Pearson’s rho**	***P*-value**	**Pearson’s rho**	***P*-value**
BMI	0.041	0.803	0.044	0.788	0.066	0.687
Body fat%	0.034	0.835	0.053	0.746	0.154	0.343
Total leukocytes	0.110	0.499	0.130	0.425	0.086	0.599
lymphocytes	0.231	0.152	0.089	0.583	0.043	0.792
Granulocytes	0.029	0.122	0.146	0.369	0.111	0.496
Monocytes[a]	0.027	0.87	0.153	0.435	0.191	0.237
Age	0.129	0.428	0.064	0.693	0.042	0.799

[a] Correlation test was performed using Spearman non parametric test; $P \leq 0.05$ is considered to be statistically significant.

Table 4.10. Associations of IL-1β before, during, and after RIF.

Variable	Before Ramadan		During Ramadan		After Ramadan	
	Pearson's rho	***P*-value**	**Spearman's rho**	***P*-value**	**Pearson's rho**	***P*-value**
TNF-α	0.293	0.088	0.265	0.124	0.429	**0.010**
IL-6	0.527	**0.000**	0.035	0.228	0.422	**0.007**
BMI	0.056	0.730	-0.308	0.054	0.042	0.796
Body fat%	0.013	0.938	-0.372	**0.018**	0.086	0.599
Total leukocytes	0.414	**0.008**	0.006	0.972	0.198	0.221
lymphocytes	0.249	0.122	0.148	0.363	0.109	0.502
Granulocytes	0.374	**0.017**	0.036	0.825	0.177	0.276
Monocytes[a]	0.137	0.285	0.003	0.986	0.120	0.188
Age	0.378	**0.016**	0.423	**0.007**	0.047	0.775

[a] correlation test was performed using Spearman non parametric test.

Table 4.11. Associations Between SBP and Circulating Cytokine Levels, and Body Weight, before, during, and after RIF.

Variable	Before Ramadan		During Ramadan		After Ramadan	
	Spearman's rho	***P*-value**	**Spearman's rho**	***P*-value**	**Spearman's rho**	***P*-value**
IL-1β	0.137	0.400	0.017	0.915	0.297	0.096
IL-6	0.099	0.544	0.088	0.591	0.195	0.199
TNF-α	**0.036**	0.813	0.231	0.126	0.351	**0.026**
Body weight	**0.047**	0.745	0.208	0.147	0.164	0.255

$P \leq 0.05$ is considered to be statistically significant.

Table 4.12. Associations between DBP and Circulating Cytokine Levels, and Body Weight, before, during, and after RIF.

Variable	Before Ramadan		During Ramadan		After Ramadan	
	Spearman's rho	***P*-value**	**Spearman's rho**	***P*-value**	**Spearman's rho**	***P*-value**
IL-1β	0.210	0.192	0.040	0.809	0.329	**0.038**
IL-6	0.294	0.066	0.007	0.966	0.354	**0.025**
TNF-α	0.028	0.853	0.036	0.825	0.168	0.271
Body weight	0.134	0.353	0.351	**0.012**	0.305	**0.031**

$P \leq 0.05$ is considered to be statistically significant.

Table 4.13. Association Between Body Weight and Body Fat %, before, during, and after RIF.

	Before Ramadan		During Ramadan		After Ramadan	
	Pearson ' r	*P*-value	Pearson ' r	*P*-value	Pearson ' r	*P*-value
Body fat %	0.380	**0.007**	0.438	**0.001**	0.445	**0.001**

$P \leq 0.05$ is considered to be statistically significant.

Participants who lost weight during RIF have lower cytokine levels (IL-6 and TNF-α), but those who lose body fat have only lower IL-6 (Table 4.14). On the other hand, subjects showing decreased total WBC during RIF have lower cytokine levels (Table 4.15).

Table 4.14. Effects of Reduction in Body Weight and Body Fat % on Cytokine Levels (pg/ml) during RIF.

Cytokine	Reduced body weight	Stable/increased body weight	*P*-value	Reduced body fat	Stable/increased Body fat	*P*-value
	n=31	**n=14**		**n=33**	**n=12**	
TNF-α (pg/ml)	45.41±43.41	67.28±80.15	0.240	58.69± 64.25	34.41±25.08	0.075
	n=27	**n=13**		**n=31**	**n=9**	
IL-6 (pg/ml)	65.67±45.78	71.77±63.06	0.729	61.00± 33.52	90.56±88.56	0.353
IL-1β (pg/ml)	3.71±5.05	3.64±4.59	0.967	3.91±5.31	2.92±2.75	0.599

$P \leq 0.05$ is considered to be statistically significant.

Table 4.15. Effects of Reduction in Total WBC and Granulocytes on Cytokine Levels (pg/ml) during RIF.

Cytokine	Reduced total WBC	Stable/ increased WBC	*P*-value	Reduced granulocytes	Stable/increased granulocytes	*P*-value
	n=33	**n=12**		**n=34**	**n=11**	
TNF-α (pg/ml)	45.00±40.61	72.08±87.90	0.163	43.85±39.45	78.09±91.16	0.085
	n=31	**n=9**		**n=29**	**n=11**	
IL-6 (pg/ml)	56.67±30.73	70.84±55.85	0.473	74.14±57.00	50.55±24.80	0.078
IL-1β (pg/ml)	3.22±4.53	5.27±5.79	0.270	3.26±4.69	4.81±5.27	0.372

$P \leq 0.05$ is considered to be statistically significant.

Table 4.16. Effects of Reduction in Monocyte and Lymphocyte Counts on Cytokine Levels during RIF.

Cytokine	Reduced monocytes	Stable/ increased monocytes	*P*-value	Reduced lymphocytes	Stable/increased lymphocytes	*P*-value
	n=26	**n=19**		**n=27**	**n=18**	
TNF-α (pg/ml)	50.88±40.73	54.05±75.51	0.857	55.74±68.94	46.94±34.12	0.619
	n=26	**n=14**		**n=28**	**n=12**	
IL-6 (pg/ml)	68.81±56.06	65.50±42.84	0.846	71.71±58.12	58.17±29.84	0.451
IL-1β (pg/ml)	5.52±6.84	2.70±3.06	0.163	3.30±3.92	4.58±6.65	0.450

$P \leq 0.05$ is considered to be statistically significant.

5. DISCUSSION

It is now well recognized that long-lasting modifications in the circadian distribution of the eating and sleeping schedule, during Ramadan fasting, result in various changes in metabolism (Al Hourani, et al., 2009). This study evaluates, to our knowledge for the first time, the effect of RIF on serum levels of the main proinflammatory cytokines, namely IL-1β, and further investigated the effect on IL-6, and TNF-α. Effects on leukocytes, blood pressure, and body composition were also evaluated. Sample homogeneity was obtained by choosing individuals from the same living condition and having closed ethnic and cultural variables. This may decrease confounders interfering with various measurements, such as changes in socioeconomic conditions affecting food choices and purchasing ability. As a result, the differences observed between measurements at all of the three study time-points should be related to fasting practice rather than to socio-economic, cultural, or ethnic variables.

Significant weight loss of 1.24 ±1.25 Kg, and significant body fat reduction (3.74±4.19 %) were observed among our subjects during Ramadan. However, body weight was recovered to baseline value one month after Ramadan. Our results are in accordance with previous findings showing significant decrease in body weight (Adlouni, et al., 1998; Al-Numair, 2006), and significant decrease in body fat (Al-Hourani and Atoum, 2007), during Ramadan.

Energy balance plays a regulatory role in body weight changes (Hussain and Leeds, 1996). Although this was not estimated in our study, some authors have associated Ramadan-related weight loss with a reduction in total energy intakes (Al-Numair, 2006; Shariatpanahi, et al., 2008), while others, have explained it by negative fluid balance during

Ramadan (Gumaa, et al., 1978; Sweileh, et al., 1992). However, weight loss observed among our study subjects is, most probably, due to reduced body fat, as evidenced by the strong positive correlation between body weight and body fat during each of the three study time-points (Table 4.13). Interestingly, while body fat is significantly increased, waist and hip circumferences are significantly decreased after Ramadan. This may allow for the possibility of the pattern of body fat redistribution after Ramadan which differs between males and females.

On the other hand, since TNF-α and IL-6 are well known inhibitors of LPL activity (Coppack, 2001), decreased TNF-α and IL-6 levels may increase LPL activity during Ramadan. This was previously evidenced by increased LPL-catalyzed reaction products such as free fatty acids, during Ramadan (Chennaoui, et al., 2009). Lipoprotein lipase is mainly synthesized in adipose tissue. It constitutes a key enzyme that hydrolyses TG in circulating TG-rich lipoproteins such as very low density lipoprotein (VLDL) and chylomicrons (Wang and Eckel, 2009). Accordingly, increased LPL activity during Ramadan contributes to reduced lipid accumulation within adipose tissue, and thus reduced body weight.

At the same time, since during fasting glucose is less available, fat assumes a greater role as substrate for energy production, and accordingly, body fat and body weight are decreased (El Ati, et al., 1995). In fact, insulin hyposecretion during Ramadan (Shariatpanahi, et al., 2008) favors a predominant lipolytic state (Chennaoui, et al., 2009) which results in increased fat oxidation observed during Ramadan (El Ati, et al., 1995).

Fasting reduces global cell proliferation rates (Varady, et al., 2007; 2008). This is evidenced in our study by the significant reduction in immune cells proliferation. Indeed, being in the reference ranges, total WBC, lymphocytes, monocytes, and granulocytes were

significantly decreased during Ramadan, and then returned to basal values one month after Ramadan, except for monocytes which significantly increased after Ramadan. These results are in accordance with previous research on healthy fasting volunteers (Maughan, et al., 2008; Unalacak, et al., 2011; Argani, et al., 2003; Sarraf-Zadegan, et al., 2000), which showed a significant decrease in total WBC during Ramadan. As a matter of fact, significant increase in monocytes after Ramadan could be related to rebound increase in postprandial activation as meal frequency increases after Ramadan (Motton, et al., 2007). In addition, leptin promotes proliferation of a variety of cells such as T lymphocytes (Fantuzzi and Faggioni, 2000). Although leptin levels were not assessed in our study, decreased leptin concentration during fasting had been reported (Ahima, et al., 1996), and could explain the anti-proliferative effect of RIF. On the other hand, decreased cytokine concentrations during Ramadan, and decreased insulin-like growth factor-1 (IGF-1) during fasting (Varady, et al., 2007), may also explain this anti-proliferative effect of RIF.

Evidence in this study also suggests that fasting is associated with decreased both systolic and diastolic blood pressures. All the participants were normotensive, which is expected given the fact that only ten of them were aged more than forty years. This anti-hypertensive effect of Ramadan is supported by many studies (Dewanti, et al., 2006; Mansi, 2007; Shariatpanahi, et al., 2008). In fact, the decrease in blood pressure during Ramadan is more probably due to reduction in body weight (Dewanti, et al., 2006). However, while DBP was positively associated with body weight, during and after Ramadan, SBP in contrast, was not associated with body weight during any of the three study time-points. Interestingly, we found positive association between SBP and TNF-α levels after Ramadan. Similarly, significant positive association was found between DBP and both IL-6 and IL-1β, after Ramadan. These results are further supported by findings form Fernandez-Real

and colleagues study (2001), where the plasma IL-6 concentration was significantly and similarly associated with systolic and diastolic blood pressures. On the other hand, Temizhan and colleagues (1999) have postulated that decreased blood pressure during Ramadan is due to the inhibition of catecholamine production. Indeed, during hunger, catecholamine inhibition and reduced venous return cause a decrease in the sympathetic tone which leads to a fall in blood pressure, heart rate, and cardiac output.

In accordance with previous research on healthy adults observing Ramadan (Aksungar, et al., 2007; Unalacak, et al., 2011), we found a significant decrease in circulating levels of all of the three investigated proinflammatory cytokines, during Ramadan fasting. According to the questionnaire, none of our subjects used any drug before, during or after Ramadan. In addition, individuals showing decreased body weight during Ramadan had lower TNF-α and IL-6 levels than their counterparts, whereas, individuals showing decreased body fat during Ramadan had lower IL-6 but not TNF-α nor IL-1β levels (Table 4.14). In fact, it was postulated that decreased body weight may play an important mechanistic role leading to reduction in circulating proinflammatory cytokines, including IL-6 and TNF-α (Corpeleijn, 2005). Mohamed-Ali and colleagues (1997) have estimated that as much as a third of total circulating concentrations of IL-6 originate from adipose tissue. This cytokine secretion activity was also attributed to visceral rather than SQ fat (Fried, et al., 1998; Fontana, et al., 2007). Although fat distribution was not assessed in our study, significant decrease in visceral rather than SQ fat was reported during RIF (Yucel, et al., 2004). Thus, decreased visceral fat might contribute to decreased IL-6 levels during Ramadan. Unexpected negative correlation was found between IL-1β levels and body fat percentage ($r = -0.372$, $P = 0.018$) during Ramadan. This is in contrast with previous findings linking adipose tissue to increased cytokine production, including IL-1β, IL-6, and TNF-α (Fain, 2006). This may

be related to decreased IL-6 levels during Ramadan, since IL-6 plays an inhibitory effect on IL-1β expression (Tilg, et al., 1994), and since 30 % of circulating IL-6 originate from adipose tissue (Mohamad-Ali, et al., 1997).

On the other hand, more than 80% of cytokine production by adipose tissue originates from nonfat cells of which macrophages are the major constituents (Fain, 2006). Additionally, weight loss is associated with a reduction in macrophage infiltration of adipose tissue (Bastard, et al., 2006). As macrophages are mainly derived from monocytes, it may be postulated that decreased cytokine levels may be indirectly related to decreased monocytes count during Ramadan. Interestingly, Motton and colleagues (2007) have reported a postprandial activation of monocytes expressing TNF-α and IL-1β in response to meals. Indeed, activation of circulating monocytes allows them to proliferate and differentiate into macrophages. This activation also allows them to be recruited by adipose tissue and other tissues. Another important observation of Motton study is the longevity of the increase in monocyte TNF-α extended to more than 8 h after each meal. This allows for the possibility that Ramadan fasting may simply decrease monocytes count by reducing their postprandial activation, as meal frequency is reduced to two meals rather than three to four meals usually consumed during non fasting periods. Accordingly, it may be postulated that decreased IL-1β and TNF-α levels might be due to the synergistic effect of both reduced body fat and decreased monocytes count during Ramadan. Additionally, decreased meal frequency during Ramadan may contribute to the cumulative decrease in cytokine levels, since postprandial hyperglycemia acutely increase circulating levels of IL-6 and TNF-α (Esposito, et al., 2002).

Given the fact that WBC are the primary source of cytokines (Parkin and Cohen, 2001), decreased circulating cytokine levels may also be attributed to the significant reduction in

circulating producing cells, including granulocytes and lymphocytes. This is illustrated in our study by reduced cytokine levels (TNF-α, IL-1β, and IL-6 levels) in individuals showing lower total WBC count during Ramadan (Table 4.15). In addition significant positive correlation was found between IL-1β and total WBC (r =0.414, *P*= 0.008) and granulocytes (r= 0.374, *P*= 0.017).

It may be instructive to also consider that decreased oxidative stress during Ramadan (Ibrahim, et al., 2008) may involve decreased ROS which play an important role in activating the transcriptional factor NF-$_{K}$B. Accordingly, fasting might inhibit the transcriptional activity of NF-$_{K}$B which is responsible for the expression of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α.

Although, homocysteine levels were not assessed in our study, evidence from previous studies suggests that decreased homocysteine levels during Ramadan (Akungar, et al., 2005; 2007) may have a profound role in the reduction of IL-6, and accordingly IL-1 and TNF-α, in view of their involvement in immune reactions within a cascade manner. In fact, cytokines can either induce or inhibit expression of each other and also regulate expression of their own receptors in a complex manner (Parkin and Cohen, 2001). Interleukin-6 for example, inhibits the expression and secretion of both TNF-α and IL-1β (Hursting, et al., 2006). This latter, in turn, induces IL-6 expression (Bruunsgaard, et al., 2000). This is evidenced in our study by the strong positive correlation between IL-1β and both IL-6 (r=0.527, *P*=0.000) and TNF-α (r=0.429, *P*=0.010).

To conclude, several factors play synergistic roles in decreasing circulating cytokine levels during Ramadan. These might include reduced adiposity; reduced postprandial activation of monocytes; reduced producing cells (WBC); reduced homocysteine and leptin levels; and reduced oxidative stress.

While previous evidence suggests an age-dependent profile of cytokine expression, our results, however, failed to find any association between IL-6 and TNF-α, and age. Interleukin-1β, in contrast, was positively associated with age. In fact, this discrepancy with previous evidence linking advanced aged with increased IL-6 and TNF-α levels (Pacifici, 1999; O'Mahony, et al., 1998; Bruunsgaard, et al., 2000; 2001) may be explained by the lower number of participants aged more than forty years. Indeed, only ten of study subjects were aged more than forty years. The use of forty years of age as cut point is based on the fact that most of age associated diseases, often, appear after this age.

The interplay between inflammation and lipid metabolism has recently been the focus of research aimed at understanding the mechanisms of atherogenesis (Fon Tacer, et al., 2007). As previously mentioned, decreased circulating IL-6 and TNF-α levels might account for increased lipolytic activity of LPL. Indeed, in case of LPL deficiency, low HDL level is caused by an insufficient transfer of surface components from VLDL to HDL (Wang and Eckel, 2009). Additionally, it has been shown that increasing LPL activity will lead to increase HDL levels (Tsutsumi, 2003). Furthermore, it is valuable to note that the increase in HDL levels during Ramadan was maintained for at least one month after Ramadan (Adlouni, et al., 1999; Mansi, 2007), and that in our study, IL-6 levels still remained depressed one month after Ramadan. Although not statistically significant, TNF-α levels also were lower one month after Ramadan. In addition, TNF-α interferes with lipid homeostasis and activates proatherogenic processes, including reduction of HDL-cholesterol and increase of LDL-cholesterol (Fon Tacer, et al., 2007). While increased HDL levels during RIF were attributed to changes in dietary patterns, as increased dietary fat was previously reported during Ramadan (Chaouachi, et al., 2007), findings from our study

raise the hypothesis that decreased IL-6 and TNF-α levels might be the most probable explanation for the anti-atherogenic effect of Ramadan fasting.

The protocol used in the present study may probably fail to give a better comparison between parameters in view of the prolonged blood sampling duration which lasted from 10 am to 4 pm. Although diurnal rhythms of IL-1β and IL-6 were markedly modified by IF in animal models (Luna-Moreno, et al., 2009), Aksungar and colleagues (2007), however, did not find any significant difference between morning and afternoon IL-6 levels, during Ramadan fasting. Accordingly, the prolonged blood sampling duration could, most probably, not affect our results. In addition, both female and male subjects were included. Indeed, the interruption of fasting during the menstrual period (5±2 days) seems to be without effect, as the data from female subjects showed similar reduction in study parameters. Even more, cytokine levels were lower in female than in male subjects, during Ramadan, though, female subjects had higher cytokine levels before and after Ramadan. In fact, female subjects were overweight (BMI=27.85±4.92), whereas male subjects were normal weight (BMI=24.15±4.34). This clearly indicates that the lower cytokine levels during Ramadan in female subjects may be attributed to differences in BMI and body fat (Table 4.1). Accordingly, it may be hypothesized that RIF results in more anti-inflammatory effect in overweight that in normal weight subjects.

Finally, this study was potentially susceptible to various limitations. In fact, serum may contain inhibitors and substances that may interfere with binding of antibodies in immunoassays to certain epitopes on the cytokine molecule, and thus generate false negatives. This issue might be overcome if we run in parallel a bioassay, which will detect the biological activity, and an immunoassay which will detect the total levels of cytokines. In addition, the pre- and post-fasting samples were not fasting. This might affect our results

since cytokine concentrations may acutely increase after meals (Esposito, et al., 2002). Another limitation of the present work is the lack of control (non fasting) group. Indeed, the control group is necessary to confirm the anti-inflammatory effect of RIF. However, incorporation of non-fasting subjects is not accessible because of the highly limited number and the cultural constraints for those people to express their uncommitment with fasting worship. Insufficient volume of serum, has also limited the investigation of cytokine levels in all volunteers.

6. CONCLUSIONS AND RECOMMENDATIONS

6. 1. Conclusions

This study provides an additional evidence for health-related benefits of RIF. Indeed, the results presented here demonstrated that RIF reduced inflammatory processes as evidenced by reduced levels of leukocytes and circulating proinflammatory cytokines (IL-1β, IL-6, and TNF-α). In fact, for the best of our knowledge, this is the first study investigating the effect of RIF on the serum levels of IL-6, IL-1β, and TNF-α together. Additionally, this is the first study investigating the effect of RIF on serum IL-1β concentrations. These proinflammatory cytokines are well known to play important mechanistic roles in lipid homeostasis, obesity-related insulin resistance, and auto-immune diseases. On the other hand, RIF effectively reduced body weight, body fat, and waist and hip circumferences. Therefore, such direct impact of fasting on cytokines provides biologically plausible mechanisms that may explain how Ramadan fasting may beneficially effect lipid and carbohydrate homeostasis as well as auto-immune diseases such as rheumatoid arthritis.

6. 2. Recommendations

It is noteworthy that RIF positively affects the inflammatory status of the body. It would be more interesting, in the future, to conduct studies aiming to explore eventual mechanisms mediating this anti-inflammatory effect of RIF. Among the hypotheses which could be subjected to experimentation are decreased cellular NF-$_{K}$B activity; decreased adiponectin, homocysteine, and leptin levels; increased natural TACE inhibitors; and increased soluble cytokine receptors. It might also prove useful to measure in parallel, the effect on anti-inflammatory cytokines, like IL-10, which constitutes a potent inhibitor of the synthesis and release of proinflammatory cytokines. In short, a comprehensive study should be conducted, in which other proinflammatory and anti-inflammatory markers should be assessed, in parallel.

In addition, this study included only healthy persons. However, it would be of great interest in future studies to examine the effects of this dietary regimen on proinflammatory markers in rheumatoid arthritis and other inflammatory diseases. Also, the recruitment of non fasting (control) participants seems necessary to confirm the anti-inflammatory effect of RIF.

Another hypothesis which should be confirmed by future studies is the contributory effect of visceral rather than SQ fat in cytokine expression and secretion. As a matter of fact, cytokine secretion during RIF should be assessed in parallel with body fat distribution assessment by computed tomography scan.

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APPENDIX A

THE CONSENT FORM

نموذج موافقة على المشاركة في البحث العلمي

عنوان الدراسة:

" تقييم تأثير الصيام المتقطع الطويل لدى مجموعة من المتطوعين الاصحاء على بعض المؤشرات الحيوية المرتبطة بالمناعة و مقاومة الالتهاب "

مقدمة:

فرض الله عز وجل صيام رمضان على كل مسلم بالغ عاقل، وقد لخص القرآن الكريم أهمية وفائدة الصيام في شهر رمضان بقوله تعالى:"**وأن تصوموا خير لكم إن كنتم تعلمون**". وأظهرت نتائج البحث العلمي أن ممارسة الصيام لدى فئات من الناس من غير المسلمين تزيد من متوسط العمر لدى هذه الفئات وتقلل من حالات الوفاة بسبب أمراض السرطان وأمراض القلب والشرايين. ونظراً لما للصيام في شهر رمضان من خصائص مميزة له عن غيره من أشكال الصيام المتبع في الأديان الأخرى، فقد هدف البحث إلى معرفة أثر الصيام في شهر رمضان المتبع لدى المسلمين على مجموعة من المؤشرات التي ترتبط بالمناعة ومنع حصول الالتهابات ومن ثم السرطان، إضافة إلى اختبار أثر الصيام على مكونات الدم وخصائصه المناعية، وكذلك أثره على وزن ومكونات الجسم.

الفحوصات والاختبارات التي سوف يتم إجراؤها: سوف يتم في هذا البحث إجراء ما يلي على الشخص المتطوع:

1. أخذ القياسات الجسمية مثل الطول والوزن ومحيط الخصر ومحيط الورك، وكذلك قياس محتوى الجسم من الدهون.
2. قياس ضغط الدم الانقباضي والانبساطي.
3. أخذ عينة دم عبر الوريد مقدارها 10 ملليلتر.

ملاحظة هامة: سيتم أخذ العينات وإجراء الفحوصات ثلاث مرات فقط على فترات متباعدة وهي: قبل رمضان بأسبوع عند الأسبوع الثالث من شهر رمضان، وبعد شهر من انتهاء رمضان.

الخصوصية والسرية

يتعهد فريق الباحثين بأنه سوف يتم التعامل مع نتائج الفحوصات المخبرية والسريرية لكل شخص بمنتهى السرية والكتمان ولن يطلع عليها أي شخص آخر غير صاحب العلاقة. كما لن يتم ذكر اسمك أو أي صفة لك معروفة في أي كتابات أو منشورات مستقبلية.

كما يُرجى العلم بأن:

1. هذه الدراسة لن تؤثر سلباً على صحتك ولن تكلفك أية أعباء مالية لإجراء الفحوصات المطلوبة ويتكفل فريق الباحثين بتغطية نفقات التحاليل المخبرية والفحوصات السريرية.
2. نتائج هذه الدراسة سيتم نشرها على شكل بحث علمي أو محاضرة أو مقال صحفي.
3. لن يتم نشر أي اسم في أي من المنشورات أو المحاضرات، وخلال جمع المعلومات سوف يتم استبدال الاسم برمز خاص وسيتعرف الباحثون فقط على الاسم الحقيقي.

الفوائد المتوقعة من البحث:

النتائج المتوقعة من هذا البحث سوف تسهم في تعميق فهم المسلم لطبيعة الصيام وأثره على الصحة وتبرز أهمية ودور شعائر الإسلام التعبدية في حفظ صحة الإنسان وتحقيق مصلحته، كما سوف يتم توظيف نتائج البحث في مجال الصحة الوقائية ضد أسباب الوفاة المباشرة مثل أمراض السرطان.

جملة الموافقة:

"لقد قرأت وفهمت بشكل كامل المعلومات المذكورة أعلاه وأوافق على المشاركة في البحث تطوعاً ودون مقابل"

اسم المشارك:..

توقيع المشارك: ....

التاريخ...

APPENDIX B
THE QUESTIONNAIRE

استبانة شخصية وصحية

الرقم المتسلسل: التاريخ:

معلومات شخصية واجتماعية

الاسم:

العنوان: تلفون:

فاكس: بريد إلكتروني:

الجنس: □ ذكر □ أنثى تاريخ الميلاد: ....

الجنسية:

الحالة الاجتماعية: □ متزوج □ أعزب

السيرة المرضية

1. هل تعاني من أي مرض؟ □ نعم □ لا

حدد نوع المرض

2. هل تعاني من أي من الأمور التالية؟

□ مرض السكري

□ مرض ارتفاع الضغط

□ ارتفاع الدهنيات في الدم

3. هل أجريت لك أية عملية جراحية من قبل؟

□ نعم متى؟ حدد نوعها:

□ لا

التاريخ الدوائي

- أذكر جميع الأدوية التي تتناولها ***حاليا*** بما فيها الأدوية التي لا تحتاج لوصفة طبية أو مكملات غذائية:

□ **مضادات الحساسية**

الكمية:__ حبة/اليوم

ما المدة (منذ متى)؟ □ سنة واحدة أو أقل□ 2-4 سنوات □ 5-9 سنوات □ 10 سنوات أو أكثر

□ **مضادات الالتهابات**

الكمية:__ حبة/اليوم ما المدة (منذ متى)؟

□ سنة واحدة أو أقل □ 2-4 سنوات □ 5-9 سنوات □ 10 سنوات أو أكثر

□ **أدوية التهاب المفاصل الروماتيزمي**

الكمية:__ حبة/اليوم

ما المدة (منذ متى)؟

□ سنة واحدة أو أقل □ 2-4 سنوات □ 5-9 سنوات □ 10 سنوات أو أكثر

□ **مكملات غذائية**: حدد النوع (مثال، Centrum)

الكمية:__ حبة/اليوم ما المدة (منذ متى)؟

□ سنة واحدة أو أقل □ 2-4 سنوات □ 5-9 سنوات □ 10 سنوات أو أكثر

□ **أدوية أخرى**: أذكرها

الكمية : __حبة/اليوم

ما المدة (منذ متى)؟ *(لكل دواء)*

□ سنة واحدة أو أقل □ 2-4 سنوات □ 5-9 سنوات □ 10 سنوات أو أكثر

التدخين: هل تدخن؟ نعم لا

كم عدد السجاير؟

تقييم تأثير الصيام المتقطع الطويل لدى مجموعة من المتطوعين الاصحاء على بعض المؤشرات الحيوية المرتبطة بالمناعة و مقاومة الالتهاب

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ملخص

لقد أظهرت الدراسات العلمية أنّ لتقليل عدد الوجبات خلال الصّيام المتقطع تأثيرا إيجابيا على مؤشرات الالتهاب والخلايا المناعية المسببة للالتهاب. لذلك هدفت دراستنا إلى تحديد تأثير الصيام المتقطع الطويل المتبع خلال شهر رمضان الكريم على مؤشرات الالتهاب و الخلايا المناعية. أجريت الدراسة خلال رمضان لعام 2009. خمسون من المتطوعين الأصحاء (21 ذكر تتراوح أعمارهم من 18 إلى 49 سنة و29 أنثى تتراوح أعمارهم من 18 إلى 51 سنة) شاركوا فيها. تمّ تجميع عينات الدم على ثلاث مراحل، أسبوع قبل رمضان و عند الأسبوع الثالث من رمضان وبعد شهر من انقضاء رمضان. عند كل مرحلة من المراحل الثلاث، تمّ قياس كل من مؤشرات الالتهاب (السايتوكينز) وخلايا الدم البيضاء واللمفاويات وحيدات النوى ومتعددة النوى والوزن والطول ومحيط الخصر ومحيط الورك ومحتوى الجسم من الدهون وضغط الدم الانقباضي والانبساطي. مقارنة مع نتائج المرحلة الأولى (قبل رمضان)، لاحظنا خلال المرحلة الثانية (عند الأسبوع الثالث من رمضان) انخفاضا في عدد خاليا الدم البيضاء واللمفاويات ووحيدات النوى ومتعددة النوى. إلى جانب ذلك، لاحظنا انخفاضاً في مؤشرات الالتهاب والوزن ومحتوى الجسم من الدهون. كذلك ضغط الدم الانقباضي و الانبساطي انخفضا خلال شهر رمضان. إنّ نتائج هذه الدراسة توضح أنّ للصيام المتقطع الطويل المتبع خلال شهر رمضان الكريم تأثيراً إيجابياً على الالتهاب.